

FABRIQUES DE TABAC REUNIES S.A.

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Plate Incorporation Mutagenicity Study of

Whole Smoke Condensate of Cigarettes

LEAR 0-17-2, LEAR 0-17-3, LEAR 2-17-2,

LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3

and of Standard Reference Cigarette 2R1

on Salmonella Typhimurium Strains TA 98 and TA 100

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ABBREVIATIONS (a,b)

=====

A	: absorbance
2-AA	: 2-aminoanthracene
2-AF	: 2-aminofluorene
AHM	: aryl hydrocarbon monooxygenase (EC 1.14.14.2)
approx.	: approximately
ART.	: article
B(a)P	: benzo(a)pyrene
BSA	: bovine serum albumin
BW	: body weight
CA	: cellulose acetate
CFU	: colony forming units
cig.	: cigarette
COEFF.	: coefficient
CONC.	: concentration
cond.	: condensate
CORREL.	: correlation
DIL. SUSP.	: diluted suspension
DIN	: Publication of the German Committee of Standards
DMSO	: dimethylsulfoxide
DPM	: dry particulate matter
EC	: enzyme code according to the "International Union of Biochemistry Commission on Enzymes"
equiv.	: equivalent
FID	: flame ionisation detector
G6P	: glucose-6-phosphate
G6PDH	: glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
.GT.	: greater than

-
- (a) in addition to those, which are explained immediately on the same page in the text, tables or figures
- (b) Units are given in accordance with SI-norms (Système International d'Unités).

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ABBREVIATIONS (continued)

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HPLC : high performance liquid chromatography
INDIV. : individual
ISH : SH-index, thiol reactivity of cigarette smoke
L : light
LD : light/dark
.LT. : less than
MC : moisture content
MMS : methyl methanesulfonate
MNNG : N-methyl-N'-nitro-N-nitrosoguanidine
N : number of individual values
NAD : nicotine adenine dinucleotide
NADP : nicotine adenine dinucleotide phosphate
2-NF : 2-nitrofluorene
no., No. : number
OW : organ weight
pH : negative decadic logarithm of hydrogen-ion concentration
prot. : protein
REGRESS. : regression
rev. : revertants
rpm : revolutions per minute
RSD : standard deviation relative to the mean in percent
RT : room temperature
RTD : resistance to draw
SE : standard error
S9 : supernatant of 9000 x g centrifugation
SPEC. : specific
SPF : specified pathogen free
TAR : TPM minus nicotine and water
TPM : total particulate matter
Tris-HCl : tris(hydroxymethyl)aminomethane hydrochloride

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ABBREVIATIONS (continued)

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U : unit
 UV : ultra-violet
 WSC-I : whole smoke condensate(s) obtained by impaction trap
 WW : wet weight
 x g : centrifugal force in terms of the constant of gravitation
 (1 x g = 9.81 m/s²)
 10Ex : x is the exponent to the base of 10

 0 : no response
 + : response
 - : not assayed

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1 SUMMARY =====

1.1 Objective

The mutagenicity of whole smoke condensate of cigarettes LEAR o-17-2, LEAR o-17-3, LEAR 2-17-2, LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3 are compared in the plate incorporation mutagenicity assay with *Salmonella typhimurium* (Ames et al., 1975). The cigarette 2R1 is used as reference.

1.2 Cigarettes

LEAR o-17-2, LEAR o-17-3, LEAR 2-17-2, LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3 are filter cigarettes belonging to project "LEAR".

The cigarette 2R1 is the internationally used standard reference cigarette produced for and used by the University of Kentucky Research Foundation.

1.3 Experimental

The experiment was performed as 2 independent assays using in each *Salmonella typhimurium* TA 98 and TA 100 as tester strains. Homogenate (S9 protein) from Aroclor 1254-induced rat liver was used for metabolic activation.

The tester strains TA 98 and TA 100 are used to detect frameshift mutagens and mutagens causing base-pair substitutions, respectively. The strains were checked and found to be in accordance with the requested characteristics.

Each condensate was assayed at doses of 0, 0.05, 0.10 and 0.15 milligrams dry condensate per plate.

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The number of revertants was used to calculate the dose-response relationship. The specific mutagenicity, defined as the regression coefficient "a" of the linear dose-response curve $y = ax + b$, was calculated as the extrapolated increase in the number of revertants per milligram of dry condensate.

1.4 Results

1.4.1 Tester strain TA 98, frameshift mutation

The specific mutagenicity in assay 1 was statistically not different to that of assay 2 for the 7 cigarettes. The mean specific and relative specific mutagenicity of both assays were:

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)	RELATIVE SPEC. MUTAGENICITY (o/o)
2R1	1536	100
LEAR o-17-2	2235	146
LEAR o-17-3	3508	228
LEAR 2-17-2	1930	126
LEAR 2-17-3	2942	192
LEAR 4-17-2	2023	132
LEAR 4-17-3	2988	195

The condensate of cigarettes with the last digit number "2" were found to be statistically significantly less mutagenic than the corresponding cigarettes with the last digit number "3". The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

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1.4.2 Tester strain TA 100, base-pair mutation

The specific mutagenicity in assay 1 was statistically not different to that of assay 2 for the 7 cigarettes. The mean specific and relative specific mutagenicity of both assays were:

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)	RELATIVE SPEC. MUTAGENICITY (o/o)
2R1	696	100
LEAR o-17-2	859	123
LEAR o-17-3	1354	195
LEAR 2-17-2	897	129
LEAR 2-17-3	1185	170
LEAR 4-17-2	863	124
LEAR 4-17-3	1000	144

The condensate of cigarettes LEAR o-17-2 and LEAR 2-17-2 were found to be statistically significantly less mutagenic, whereas the cigarette LEAR 4-17-2 was only numerically less mutagenic than the corresponding cigarettes with the last digit number "3". The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

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1.5 Conclusion

For frameshift mutation as well as for base-pair substitution the condensate of cigarettes with the last digit number "2" are less mutagenic than the corresponding cigarettes with the last digit number "3". The condensates of all cigarettes of project "LEAR" are more mutagenic than that of standard reference cigarette 2R1.

I N B I F O
Institut für biologische
Forschung GmbH

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2 RESPONSIBILITY

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Study Director:

.....
Dr.rer.nat. F. Tewes
Biologist (Diplombiologe)

Analytical Chemistry:

.....
Dr.rer.nat. M. Speck
Chemist (Diplomchemiker)

Biometry:

.....
H. Gugel
Mathematician (Diplommathematiker)

Quality Assurance:

.....
E. Römer
Biologist (Diplombiologe)

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3 CIGARETTES
=====3.1 General Specifications

Test substances: whole smoke condensates of cigarettes
prepared by impaction trap

Code of cigarettes

4 cigarettes from
project "LEAR":

LEAR o-17-2
LEAR o-17-3
LEAR 2-17-2
LEAR 2-17-3
LEAR 4-17-2
LEAR 4-17-3

1 standard reference
cigarette:

2R1

Date of receipt at INBIFO

LEAR cigarettes:

28.Oct.81

2R1:

Apr.78

Source

LEAR cigarettes:

FABRIQUES DE TABAC REUNIES S.A.
CH-2003 Neuchatel
Switzerland

2R1:

PM, USA

Amount

LEAR cigarettes:

1000 each, except o-17-2 (980)

2R1:

- (taken from INBIFO stock)

Packing

LEAR cigarettes:

20 cigarettes/hard box

2R1:

20 cigarettes/soft box

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Storage and conditioning

Main storage

Walk-in cold room
(room R911):

approx. 4 degrees centigrade,
relative humidity uncontrolled
(2R1 only)

Conditioning

Conditioning room
(room R326):

cigarettes taken out of their
packaging and deposited in
open racks in upright position

stored for at least 4 d prior
to use at approx. 22 degrees
centigrade, approx. 60 o/o
relative humidity

Selection (a)

Weight (g/cig)

assay 1

assay 2

LEAR o-17-2:

1.00 to 1.06

0.99 to 1.05

LEAR o-17-3:

1.00 to 1.06

1.00 to 1.06

LEAR 2-17-2:

0.99 to 1.05

0.98 to 1.05

LEAR 2-17-3:

1.07 to 1.13

1.04 to 1.11

LEAR 4-17-2:

1.00 to 1.06

0.99 to 1.05

LEAR 4-17-3:

0.98 to 1.04

0.98 to 1.04

RTD (mm H2O):

no selection

3.2 Supplier's Specifications

Specifications of cigarettes:

see TABLE A

Physical properties and
chemical composition of
filler of cigarettes:

see TABLE B

Specifications of filters
of cigarettes:

see TABLE C

Smoke components per
cigarette:

see TABLE D

(a) selected by personnel of Microbiology lab in order to use
cigarettes within a reasonable range of weight

CIGA- RETTE	SPECIFIED PARAMETERS						
	TOTAL CIG. WEIGHT	FILTER AND PAPER WEIGHT	TOBACCO WEIGHT (a)	TOTAL CIG. LENGTH	DIAMETER	RTD	STATIC BURNING TIME
	(029) (mg/cig.)	(030) (mg/cig.)	(031) (mg/cig.)	(-) (mm)	(025) (mm)	(033) (mm H2O)	(-) (min/40 mm)
2R1	1194	110	1084	85	7.96	81	13.7
LEAR 0-17-2	1047	227	820	84	7.93	97, 95	-
LEAR 0-17-3	1025	223	797	84	7.98	110, 12	-
LEAR 2-17-2	1049	222	827	84	7.96	94, 101	-
LEAR 2-17-3	1097	232	860	84	7.91	110, 126	-
LEAR 4-17-2	1050	229	821	84	7.96	101, 104	-
LEAR 4-17-3	1027	223	804	84	7.95	111, 124	-

TABLE A

SPECIFICATIONS OF CIGARETTES

specifications and method numbers (b) provided by the supplier

(a) at o/o FC (= moisture found)

(b) method numbers given in brackets just below parameters

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CIGA- RETTE	FILLER				
	TOTAL ALKALOIDS	REDUCING SUGARS	NITRATE NITROGEN	AMMONIA NITROGEN	NITROGEN TOTAL
	(074)	(075)	(076)	(077)	(079)
	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)
2R1	1.98	10.5	0.20	0.09	2.17
LEAR 0-17-2	1.93	7.1	0.26	0.33	-
LEAR 0-17-3	2.49	0.0	0.50	0.51	-
LEAR 2-17-2	1.63	7.9	0.10	0.22	-
LEAR 2-17-3	1.60	0.6	0.02	0.16	-
LEAR 4-17-2	1.76	7.5	0.16	0.27	-
LEAR 4-17-3	2.05	0.0	0.28	0.27	-

TABLE B

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF FILLER OF CIGARETTES
 specifications and method numbers (a) provided by the supplier

(a) method numbers given in brackets just below parameters

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CIGA- RETTE	FILLER									
	CHLORIDE	ASHES	POTASSIUM	CALCIUM	MAGNESIUM	HOT WATER SOLUBLES	TOBACCO pH	TOBACCO MOISTURE CONC.	EQUILIBRIUM MOISTURE CONC.	FILLING POWER CV (a)
	(112)	(113)	(114)	(115)	(371)	(305)	(111)	(071)	(122)	(120)
	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)	(o/o)	(ml/10 g)
2R1	0.66	14.8	-	-	-	59.0	5.5	-	12.6	-
LEAR 0-17-2	0.72	14.0	4.23	2.78	0.51	51.8	-	12.6	10.6	36
LEAR 0-17-3	0.71	18.5	4.54	3.71	0.59	44.35	-	10.8	9.1	44
LEAR 2-17-2	0.60	13.8	3.79	2.53	0.44	42.26	-	12.5	11.6	31.1
LEAR 2-17-3	0.73	19.9	5.09	3.74	0.45	32.63	-	12.1	9.7	42.6
LEAR 4-17-2	-	-	-	-	-	51.39	-	12.8	11.7	40.7
LEAR 4-17-3	0.58	16.5	4.25	3.64	0.52	38.99	-	12.6	13.5	45.9

TABLE B (continued)

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF FILLER OF CIGARETTES

specifications and method numbers (b) provided by the supplier

(a) at 12 o/o MC (moisture content)

(b) method numbers given in brackets just below parameters

1688709202

CIGARETTE	FILTER TYPE	MATERIAL	LENGTH (mm)	TIPPING PAPER LENGTH (mm)	RTD (mm H2O)
2R1	- (a)	-	-	-	-
LEAR 0-17-2	S	CA	20	24	62
LEAR 0-17-3	S	CA	20	24	63
LEAR 2-17-2	S	CA	20	24	66
LEAR 2-17-3	S	CA	20	24	62
LEAR 4-17-2	S	CA	20	24	66
LEAR 4-17-3	S	CA	20	24	61

TABLE C

SPECIFICATIONS OF FILTERS OF CIGARETTES

specifications provided by the supplier

(a) no filter

2026048892

CIGA- RETTE	PUFF COUNT	SMOKE COMPONENTS									
		TPM	WATER IN TPM	DPM	NICO- TINE	TAR	CO	NO	HCN	ALDE- HYDES	ISH
		(086)	(083)	(080)	(084)	(085)	(-)	(081)	(082)	(088)	(089)
	(1/cig.)	(mg/ cig.)	(mg/ cig.)	(mg/ cig.)	(mg/ cig.)	(mg/ cig.)	(mg/ cig.)	(mg/ cig.)	(ug/ cig.)	(mg/ cig.)	(o/o)
2R1	13.1	48.4	4.9	43.5	3.32	40.2	25.4	0.39	453	3.31	71
LEAR 0-17-2	9.0	18.4	1.8	16.6	-	-	14.1	0.27	204	1.25	-
LEAR 0-17-3	9.2	14.5	0.9	13.6	-	-	12.1	0.45	143	1.07	-
LEAR 2-17-2	8.9	18.0	1.8	16.3	-	-	16.2	0.11	186	1.28	-
LEAR 2-17-3	9.5	17.2	1.1	16.1	-	-	15.4	0.06	149	1.33	-
LEAR 4-17-2	9.4	19.2	1.8	17.5	-	-	18.1	0.18	219	1.23	-
LEAR 4-17-3	9.0	16.8	1.0	15.8	-	-	15.3	0.27	144	1.19	-

TABLE D

SMOKE COMPONENTS PER CIGARETTE

specifications and method numbers (b) provided by the supplier

(a) method numbers given in brackets just below parameters

2026048893

4 METHOD =====

4.1 Chronological Tables (see FIGURES A and B)

4.2 Condensate Preparation, Storage and Analyses

4.2.1 Preparation of whole smoke condensate by impaction trap (WSC-I)

Principle: mechanical open-end smoking to a defined butt length in automatic negative pressure (vacuum pump) smoking machine, condensate collection in impaction trap

Time: 7 or 8 d before plate incorporation assay

Sample material and quantity: cigarettes (see 3 TEST SUBSTANCES), 3o cigarettes/condensate

Number of condensates prepared: 4 batches of each type of cigarette

Equipment

Smoking machine

Type: automatic INBIFO smoking machine

Number of machines: 1

Machine no.: oo25

Loading of cigarettes: manually into the cigarette holding device up to a depth of $9 + 1$ mm in accordance with DIN 1o24o (a)

Lighting of cigarettes: automatically with an iodine spot lamp adjusted in a gold plated focal mirror

Ejection of cigarettes: automatically at butt length of 32 to 28 mm for filter cigarettes and 23 mm for filterless standard reference cigarette (automatic scanning with infrared photodiode)

(a) Maschinelles Abrauchen von Zigaretten und Bestimmung des Rauchkondensats, DIN 1o24o, April 1978

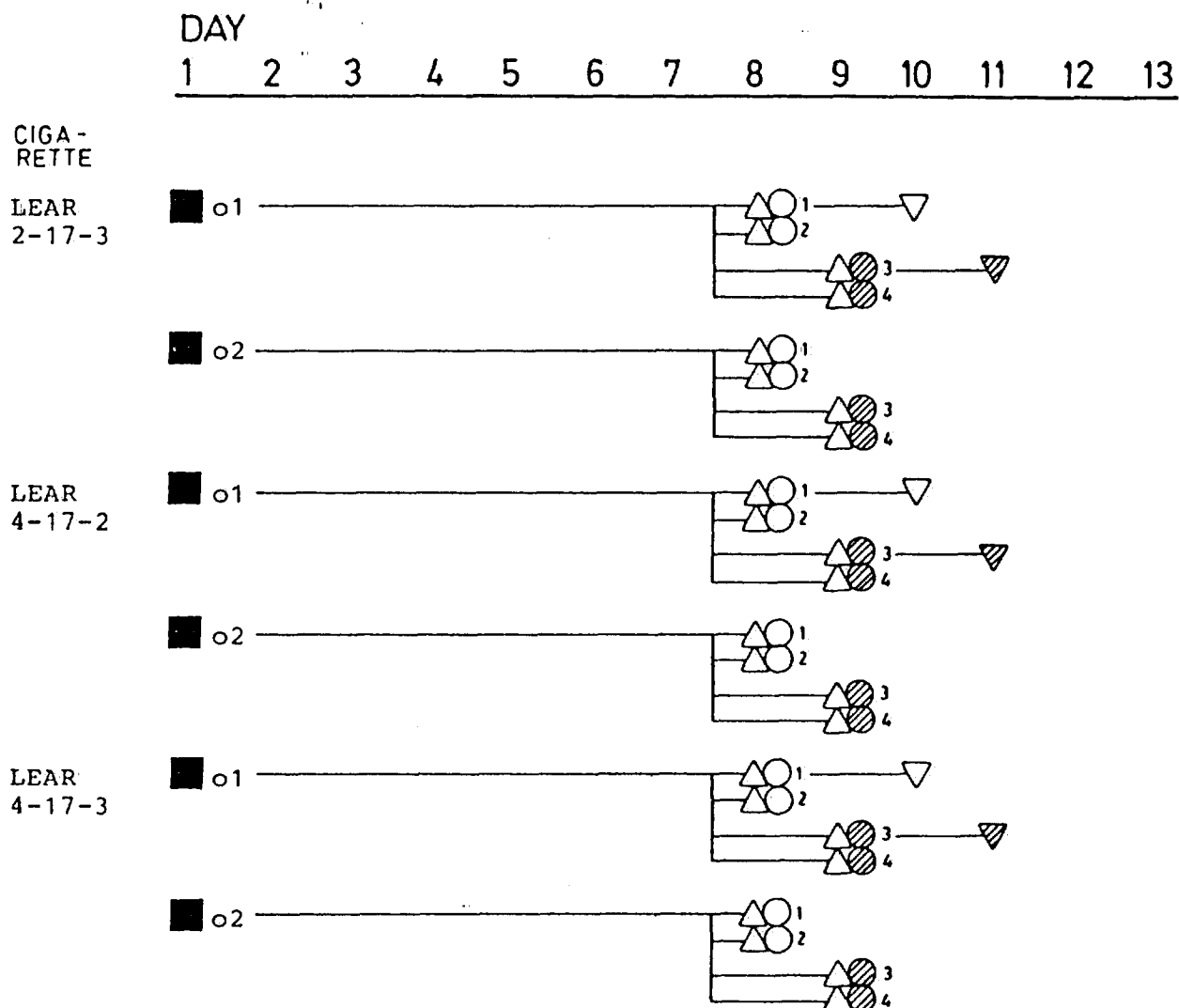


FIGURE: A (continued)

CHRONOLOGICAL TABLE, ASSAY 1

WSC-I preparation (■), dilution of WSC-I suspension (Δ), mutagenicity assay with TA 98 (○) and with TA 100 (⊗) and reversion assay with TA 98 (▽) and with TA 100 (∇) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 2.Nov.81

2026048896

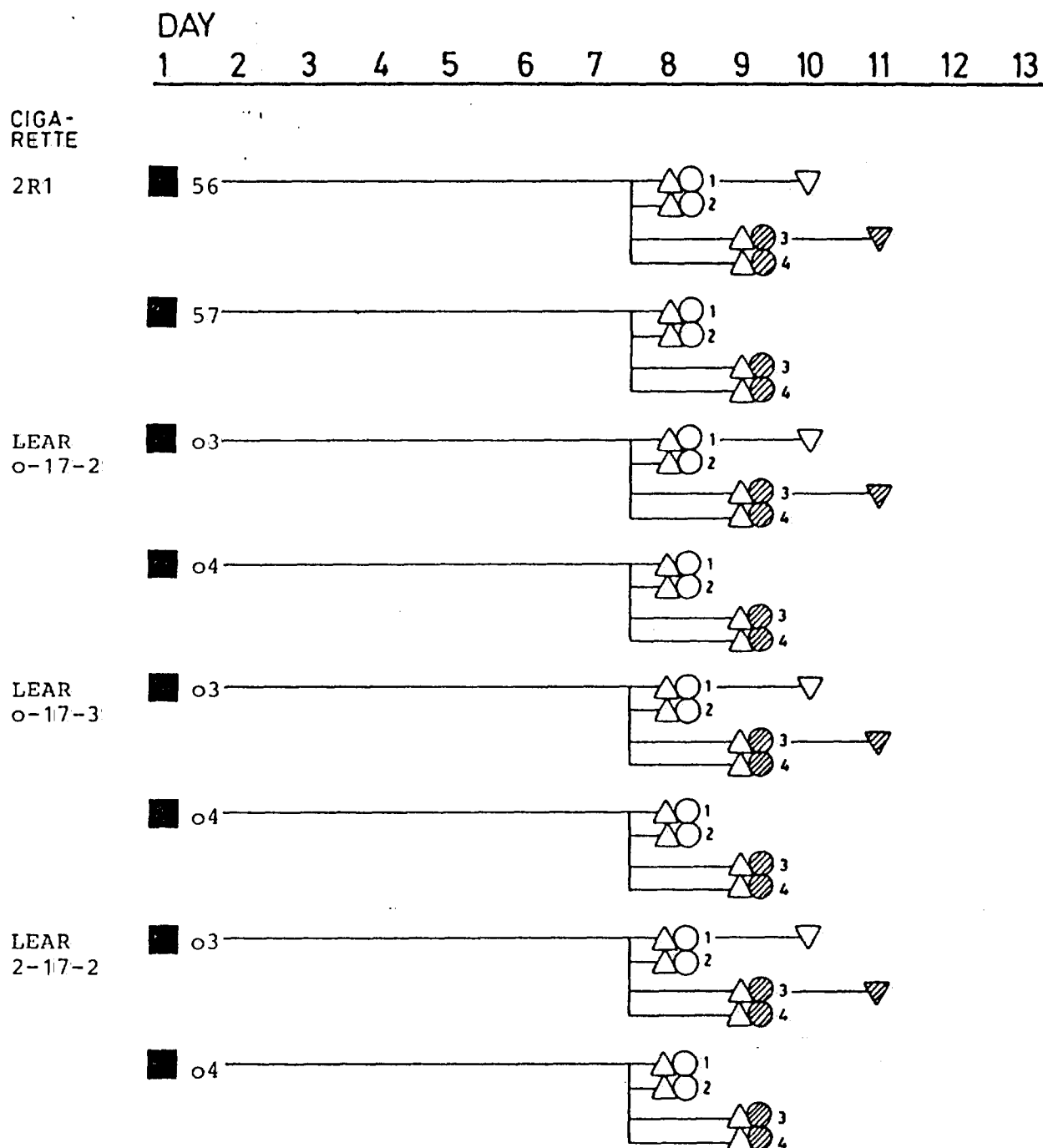


FIGURE B

CHRONOLOGICAL TABLE, ASSAY 2

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (⊗) and reversion assay with TA 98 (▽) and with TA 100 (▽) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 17.Nov.81

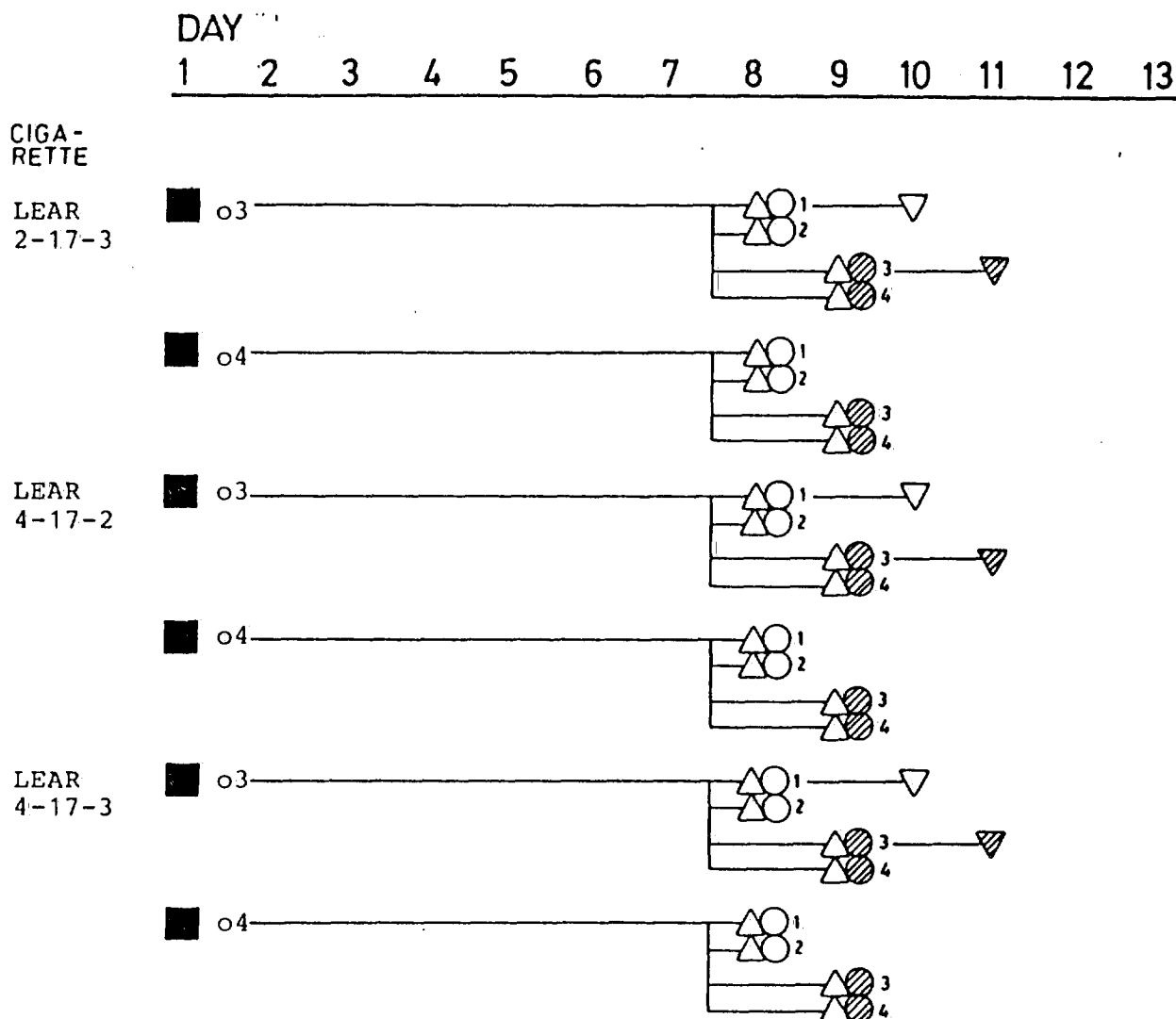


FIGURE B (continued)

CHRONOLOGICAL TABLE, ASSAY 2

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (⊗) and reversion assay with TA 98 (▽) and with TA 100 (▼) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 17.Nov.81

2026048898

Vacuum pump: rotary valve pump, Medvak MP 1,
Pfeiffer GmbH,
D-6330 Wetzlar

Rotameter: rotameter L 4/160,
Rota KG,
D-7867 Wehr/Baden

Soap-film flowmeter: Faust GmbH,
D-5000 Köln 90

Impaction trap

Type: glass "Impaction trap for cigarette
smoke condensate collection" according
to PM (see FIGURE C),
Faust GmbH,
D-5000 Köln 90

Capillary: length: 5 mm
bore: 0.4 mm

Mode of installation
of the impaction trap
insert: distance of 0.5 mm between capillary
tip and wall of flask calibrated with
0.5 mm thick teflon sheet spacer

Connection of impaction
trap to smoking machine: so that the impaction trap lies
horizontally

Procedure

Puffs/cigarette: see TABLE 1

Puff frequency/cigarette: 1 puff/min

Puff duration: approx. 2 s minus time for change
of position

Puff volume: 35 ml
parameter checked and regulated
during condensation with rota-
meter or soap-film flowmeter

Scientific version: 14.Jan.81
Text version: 10.Aug.82

2026048899

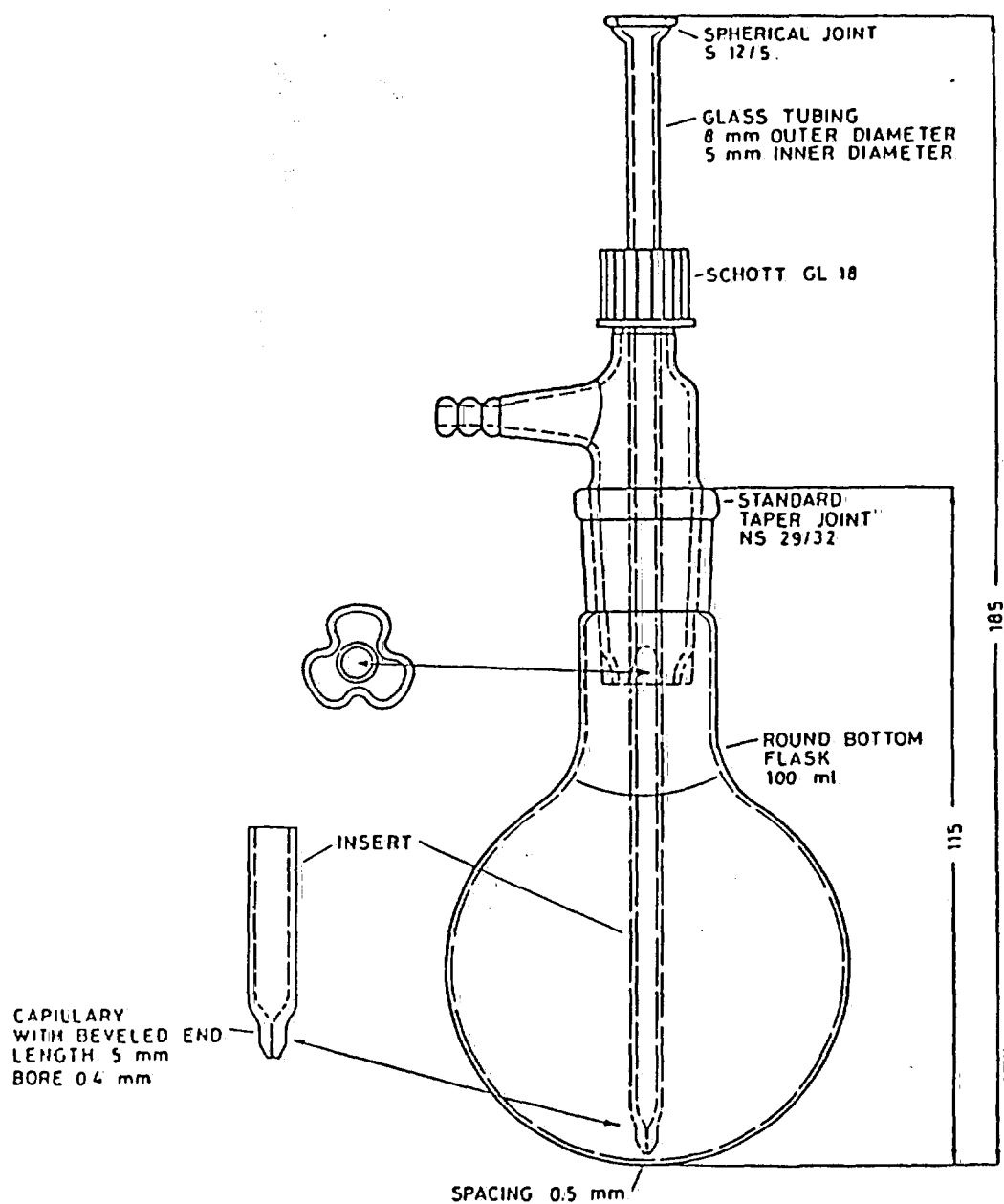


FIGURE C

IMPACTION TRAP

2026048900

4.2.2 Preparation and storage of WSC-I suspension

Principle: suspension of WSC-I in DMSO by sonication

Time: immediately after WSC-I preparation

Sample material and quantity: total WSC-I prepared from 30 cigarettes with 1 smoking machine

Equipment: sonication water bath, Sonorex RK 100, Bandelin KG, D-1000 Berlin

Chemicals and reagents: DMSO, for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure: WSC-I washed out of impaction trap 6 times with approx. 3 ml portions of DMSO repeatedly after sonication (water bath) for approx. 3 min, washings transferred to a volumetric flask and filled up to 25 ml with DMSO

amount of WSC-I calculated from weight of impaction trap before and immediately after condensate preparation

amount of dry condensate (a) calculated from WSC-I and water concentration of suspension determination: see 4.2.3

determination of nicotine concentration: see 4.2.4

(a) The terms "moist condensate" and "dry condensate" are in accordance with DIN 10240 ("Maschinelles Abrauchen von Zigaretten, Bestimmung des feuchten und des trockenen Rauchkondensates").

storage of WSC-I suspension:
in the dark, in sterile brown glass
bottles, 4 degrees centigrade

labeling of the bottles:
study no.,
batch no. (a),
dry condensate concentration (g/l),
date of condensate preparation

Scientific version: 15.Jan.81
Text version: 1.Oct.81

4.2.3 Determination of water concentration

Principle: titration according to Karl Fischer

Time: within 48 h after preparation of
WSC-I suspension

Sample material and quantity: WSC-I suspension (DMSO suspension),
1 ml, 2 determinations/suspension

Results expressed in: g/l

Equipment: Karl Fischer-Titrator E452,
Deutsche Metrohm GmbH,
D-7024 Filderstadt

Chemicals and reagents: Karl Fischer solution, no. 9248,
methanol, no. 6012,
DMSO, no. 2950,
E. Merck,
D-6100 Darmstadt 1

(a) Batch number consists of cigarette short code, type of condensate, consecutive number, date of condensate preparation, for example: 7A (I)/10/081280 (e. g.: code: 7A, condensate: I, batch no.: 10, date: 8.Dec.80).

Procedure

Titration: 1 ml DMSO mixed with 4 ml methanol in the reaction vessel of the titrator and titrated with Karl Fischer solution to determine the water content of DMSO. Afterwards 1 ml cigarette smoke condensate suspended in DMSO titrated in the same way

Computation: for computation the titer of the Karl Fischer solution determined by titration of a mixture of 1 ml DMSO and 4 ml methanol with a known amount of water, e. g. 10 mg

Detection limit: 0.5 g H₂O/l

Reproducibility (rel. standard deviation): 2.8 o/o (10 g H₂O/suspension, N = 8)

Scientific version: 2.Jun.80

Text version: 23.Aug.82

4.2.4 Determination of nicotine concentration

Principle: gas chromatography after extraction with dichloromethane

computer integration of peak areas

Time: within 48 h after preparation of WSC-I suspension

Sample material and quantity: WSC-I suspension (DMSO suspension), 1 ml, 2 determinations/suspension

Results expressed in: g/l

2026048903

Equipment:

gas chromatograph: HP 5710 A,
detector: FID,
automatic sampler: HP 7671 A,
laboratory data system: HP 3351 A,
Hewlett-Packard GmbH,
D-6000 Frankfurt/Main

recorder: Servogor 210,
Metrawatt GmbH,
D-8500 Nürnberg

Chemicals and reagents:

nicotine, no. 77635,
Serva Feinbiochemica,
D-6900 Heidelberg

quinoline, no. 802407,
dichloromethane, no. 822271,
DMSO, no. 2950,
sodium hydroxide, no. 5594,
sulfuric acid (0.2 mol/l),
no. 9074,
E. Merck,
D-6100 Darmstadt 1

nitrogen,
hydrogen,
air (synthetic),
Linde AG,
D-5000 Köln 50

Procedure

Extraction:

addition of 1 ml of the internal
standard solution (0.5 mg quinoline/
ml 0.1 mol/l sulfuric acid), 1 ml
sodium hydroxide (200 g/l) and 10 ml
dichloromethane to 1 ml WSC-I
suspension, after agitation
(5 min) and centrifugation
(approx. 7.8×10^3 m/s² (= 800 x g),
5 min, approx. 10 degrees centi-
grade), injection of 1 µl of the
lower organic phase into the gas
chromatograph

2026048904

Gas chromatography

Column: 2 m x 1/8 inch outer diameter,
stainless steel

Column packing: 10 o/o Apiezon L and 10 o/o KOH on
Chromosorb W-AW DMCS, 80 to 100 mesh

Carrier gas and
flow rate: nitrogen, 30 ml/min

Oven temperature
(degrees centigrade): 175

Injection port
temperature
(degrees centigrade): 200

Detector temperature
(degrees centigrade): 200

Computation: for computation 1 ml of a standard
solution (1 mg nicotine and 0.5 mg
quinoline/ml 0.1 mol/l sulfuric
acid) diluted with 1 ml DMSO and
extracted as described above

Detection limit: 0.02 g/l

Recovery: 98.5 o/o

Reproducibility
(rel. standard deviation): 0.5 o/o (1 g nicotine/l, N = 10)

Scientific version: 2.Jun.80
Text version: 29.Sep.81

2026048905

4.2.5 Dilution of WSC-I suspension

Principle: dilution of WSC-I suspension to defined dry condensate concentration

Time: 7 d after WSC-I preparation

Sample material and quantity: WSC-I suspension, approx. 3 ml

Equipment: Sonifier B-15, titanium microtip, Branson, Danbury, USA

Chemicals and reagents: DMSO for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure: prior to dilution: sonication of WSC-I suspension for 20 s with 10 s interval, 60 o/o power (sonifier B-15)

2 dilutions per WSC-I suspension

Scientific version: 15.Aug.80

Text version: 1.Oct.81

4.2.6 Determination of bacterial contamination of test substance

Principle: determination of bacterial contamination of test substance assayed for mutagenicity in the plate incorporation assay

detection limited to aerobic bacteria growing on minimal-glucose agar plates

2026048906

Time: during plate incorporation mutagenicity assay

Sample material and quantity: test substance (highest dose/plate)

Results expressed in: colony forming units/plate

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

petri dishes: no. 1029,
100 mm x 15 mm, polystyrole,
sterilized,
Falcon,
Oxhard, CA. 93030, USA

colony counter (manual): Colony
Star 2,
Funke-Gerber,
D-1000 Berlin

Chemicals and reagents: top agar and minimal-glucose agar,
composition: see 5.6 Plate Incorporation Mutagenicity Assay

Procedure: top agar and test substance mixed by
rotation and poured on minimal-glucose agar
2 plates/sample

incubation of 2 inoculated plates
at 37 degrees centigrade, manual
counting of colonies after 2 d of
incubation

Scientific version: 26.Jul.79
Text version: 29.Sep.81

2026048907

4.3 Preparation of Aroclor 1254-Induced Rat Liver Homogenate

4.3.1 Animals

Species: albino rat

Strain: Sprague Dawley (caw-ico-wiga)

Type of breeding: outbred

Sex: male

Microbiological
conditions of breeding: SPF

Breeder: WIGA Versuchstierzucht,
Willi Gassner GmbH und Co. KG,
D-8741 Sulzfeld

Transport containers: special filter cartons

INBIFO animal supply number: 120

Number of rats: required: 20
applied: 19

Date of shipment: 4.Aug.81

Age of rats (days): arrival: 45 \pm 2,
1st application: 49 \pm 2

Body weight (grams): arrival: 186.8 \pm 1.5
administration: 206.6 \pm 1.7
section: 220.0 \pm 2.8

Acclimatization period (days): 4

Text version: 24.Aug.81

2026046308

4.3.2 Animal housing

Animal room: INBIFO main laboratory building,
barrier area SPF, room R513

Construction and interior: windowless
floors, walls and ceilings as well
as fixtures coated with epoxy resins

Microbiological conditions: SPF-barriered animal care

Conditioning and
ventilation: 100 o/o fresh air, delivered from a
50 m high air inlet stack, approx.
15 changes/h
filter: HEPA filter

Room temperature
(degrees centigrade): 22 ± 1

Relative humidity (o/o): 55 ± 10

Light: time cycle:
LD 12:12, L 7.00 to 19.00
source:
dampened "daylight" fluorescent lamps,
Universal Weiss,
Osram GmbH,
D-8000 München 1
intensity in cages:
approx. 20 to 100 Lux (a)

Cages: polycarbonate cages (Makrolon), type 3
base area: 43 cm x 17 cm
height: 15 cm

(a) higher intensity during handling of animals and cleaning
of rooms

Cage lids: stainless steel lids with overhead
hoppers

length: 44 cm

width: 23 cm

Bedding material: autoclaved granulated wood

sterilization:

15 min at 134 degrees centigrade

pressure: 2.03×10^5 Pa

replacement of bedding material:

2 times/week during application

Animals per cage: 2

Scientific version: 15.Jan.81

Text version: 29.Sep.81

2026048910

4.3.3 Food and drinking water

Food: autoclaved fortified pellet food,
16 mm long, cylindrical pellets,
"HERILAN MRH-HALTUNG für Mäuse,
Ratten und Hamster" (a) (see PAGE
4-19),
H. Eggersmann KG,
D-3260 Rinteln/Weser
sterilization:
5 min at 120 degrees centigrade
pressure: 1.01×10^5 Pa
drying: 15 min

Food supply: ad libitum from stainless steel
hoppers in cage lid, food removed
12 h before sacrifice

Water: autoclaved tap water (a)

Water supply: ad libitum from 250-ml DIN glass
bottles, with stainless steel sipper
tubes, water changed 2 times/week

Scientific version: 15.Jan.81
Text version: 9.Dec.81

(a) random samples of all autoclaved batches of food and of drinking
water microbiologically investigated

HERILAN MRH-HALTUNG

Alleinfutter für die Haltung ausgewachsener Mäuse,
Ratten und Hamster

Abpackungen: 50-, 25-kg-Papiersäcke oder Papiersäcke mit Polyäthyleneinlage

Pressung: 16 mm, 10 mm, rund oder eckig

Energiegehalt: 10 500 joule umsetzbare Energie / kg Fertigfutter

Gehalt an Rohnährstoffen: (% der lufttrockenen Substanz)			Gehalt an Mineralien und Spurenelementen: (% der lufttrockenen Substanz) g/kg Fertigfutter	
Rohprotein	16		Ca	0,80
Rohfett	4		P	0,70
Rohfaser	6		Na	0,25
			K	1,00
			Mg	0,40
			Fe	min. 0,15
			Mn	min. 0,08
			Cu	min. 0,02
			Zn	min. 0,05

Vitaminzusatz pro kg Fertigfutter			Durchschnittlicher Gehalt an Aminosäuren: (Angaben in g AS / kg Futter)	
	Normalfutter	Fortified-Futter		
Vitamin A	10 000 I.E.	20 000 I.E.	Alanin	3,2
Vitamin D ₃	400 I.E.	600 I.E.	Arginin	22,3
Vitamin E	150 mg	200 mg	Asparaginsäure	4,5
Vitamin K ₃	10 mg	15 mg	Cystin	15,4
Vitamin C	100 mg	150 mg	Glutaminsäure	11,3
Vitamin B ₁	20 mg	30 mg	Glycin	18,1
Vitamin B ₂	30 mg	60 mg	Histidin	8,2
Vitamin B ₆	20 mg	30 mg	Iso-Leucin	12,6
Vitamin B ₁₂	30 mcg	40 mcg	Leucin	24,0
Ca-Pantothenat	40 mg	60 mg	Lysin	8,6
Nikotinsäureamid	40 mg	60 mg	Methionin	3,1
Cholinchlorid	1 000 mg	1 200 mg	Phenylalanin	3,3
Folsäure	5 mg	6 mg	Prolin	4,3
Inosit	5 mg	10 mg	Serin	2,9
Biotin	80 mcg	100 mcg	Threonin	14,0
			Tryptophan	5,1
			Tyrosin	16,3
			Valin	17,6

SPECIFICATIONS BY SUPPLIER

4.3.4 Administration of Aroclor 1254

Principle: intraperitoneal injection

Time: 5 d prior to sacrifice

Sample material and quantity: rats, 19

Equipment: sonifier: cell disruptor B-15,
titanium microtip,
Branson,
Danbury, USA

syringes: 2 ml, sterile, no. 9410002,
cannula: sterile, no. 9410118,
Hirtz and Co.,
D-5000 Köln 51

Chemicals and reagents: Aroclor 1254,
Dr. S.u.I. Ehrenstorfer,
D-8900 Augsburg

corn oil: Mazola,
Maizena GmbH,
D-7100 Heilbrunn

Procedure

Preparation: Aroclor emulsified in corn oil
by sonication (5 x 10 s, 0 de-
grees centigrade) to a concen-
tration of 200 mg/ml

Dose: 500 mg/kg BW

Scientific version: 25.Jan.80
Text version: 29.Sep.81

2026048913

4.3.5 Preparation of tissue homogenate supernatant (S9 fraction)

Principle: mechanical grinding and centrifugation of tissue

Time of sacrifice: 5 d after administration of Aroclor

Sample material and quantity: livers, 19 pooled

Final product: Aroclor 1254-induced rat liver homogenate, batch no. 81.A

Equipment: Potter-Elvehjem glass homogenizer with motor-driven teflon pestle, Braun Melsungen GmbH, D-3508 Melsungen

Multifix, model M 80, Alfred Schwinher, D-7070 Schwäbisch-Gmünd

centrifuge: Sorvall RC-5B, rotor: SS-34, Du Pont Instruments, D-6350 Bad Nauheim

freezer: Forma Scientific, no. 8218, Forma Scientific, Marietta, Ohio, USA

tubes: polycarbonate, no. 3137, polypropylene, no. 3138, Du Pont Instruments, D-6350 Bad Nauheim

polypropylene, no. 3810, Netheler and Hinz, D-2000 Hamburg 63

glassware and equipment in contact with homogenate precooled and sterile

Chemicals and reagents: potassium chloride, no. 4936, E. Merck, D-6100 Darmstadt 1

2026048914

Procedure: according to Ames et al., Mutation Research 31: 347-364 (1975)

killing:
decapitation, followed by 30 s
exsanguination

removal of organs:
sterile dissection after soaking
the fur with ethanol (700 ml/l),
short submersion of removed organs
in 150 mmol/l potassium chloride,
pH 7.0, determination of WW

homogenization:
after addition of 3 volumes of 150
mmol/l potassium chloride, pH 7.0,
to the original organ weight (1 g
equiv. to 1 ml) mechanical grinding
in Potter-Elvehjem apparatus with
motor-driven teflon pestle, approx.
200 rpm

centrifugation:
 8.83×10^4 m/s² (= 9000 x g),
10 min, 4 degrees centigrade

storage of supernatant
(S9 fraction):
minus 80 degrees centigrade,
1-ml and 5-ml aliquots

Scientific version: 7.Dec.80

Text version: 30.Dec.81

4.4 Analyses of Rat Liver Homogenates

4.4.1 Determination of protein concentration (Biuret method)

Principle: photometric determination of a
dye complex formed between pep-
tide bonds and the Biuret rea-
gent

2026048915

Time: 26.Nov.81

Sample material and quantity: S9 fraction, 1o ul

Results expressed in: g/l

Equipment: ABA-1oo Bichromatic Analyzer,
Abbott Laboratories, Diagnostics
Division,
So. Pasadena, California 91o3o, USA

Chemicals and reagents: test set Total protein (Biuret
method), no. 124281,
standard protein: Precinorm S,
no. 125121,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

Procedure: according to Weichselbaum, T.E.,
Amer. J. Clin. Path. 16: 4o (1946),
adapted by INBIFO to the bichro-
matic analyzer

photometric determination:

wavelength 1: 55o nm
wavelength 2: 65o nm

each sample determined in dupli-
cates

standard curve: see FIGURE D

Scientific version: 28.Mar.79
Text version: 27.Nov.81

4.4.2 Determination of aryl hydrocarbon monooxygenase (EC 1.14.14.2) activity-----

Principle: fluorometric determination of 3- and
9-OH-B(a)P formed during the incuba-
tion of B(a)P with tissue extracts
and separated after incubation by
HPLC

2026048916

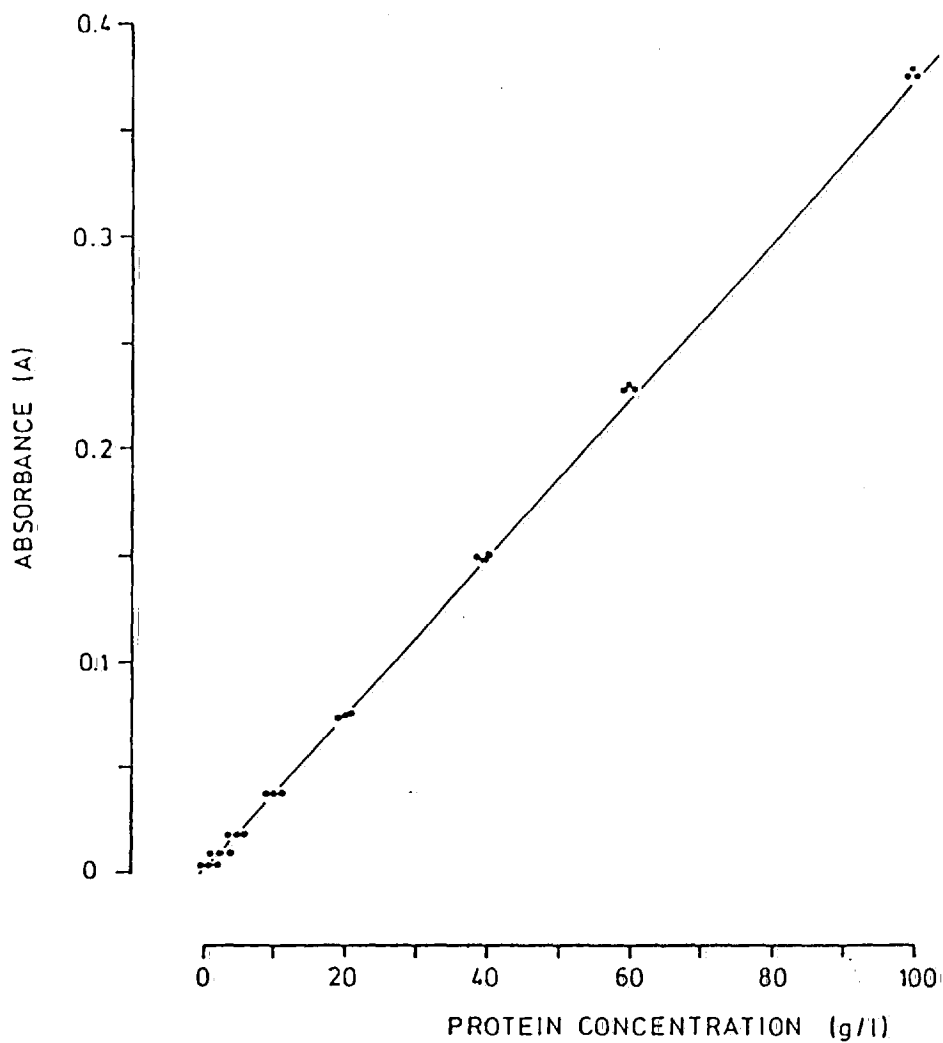


FIGURE D

PROTEIN STANDARD CURVE, BIURET METHOD

Scientific version: 28.Mar.79

2026048917

Time: 26.Nov.81

Sample material and quantity: liver: S9-fraction,
approx. 6 ug of protein

Results expressed in: U/mg protein
(1 U = (1 nmol 3- plus 9-OH-B(a)P)/h)

Equipment: rotary mixer:
Cenco Instrumenten,
Breda, The Netherlands

high pressure liquid chromatograph:
model 1084 B,
Hewlett Packard,
D-7500 Karlsruhe

column: RP-18, 250 mm x 4.6 mm,
Knauer,
D-6370 Oberursel

incubation vessels: no. 611-52,
Sovirel,
Le Vallois Perret, France

injection vial: no. 80184, 11.5 mm
x 325 mm,
Müller und Müller Glaswarenfabrik,
D-3450 Holzminden

spectrofluorometer: model 650-10S,
Perkin Elmer GmbH,
D-7770 Überlingen

shaking water bath: no. 3047,
Köttermann,
D-3165 Hänigsen

centrifuge: Sorvall RC-5B, rotor:
SS-34,
polyallomer tubes, no. 3128,
Du Pont Instruments,
D-6350 Bad Nauheim

Chemicals and reagents: magnesium chloride, no. 5833,
acetonitrile, no. 30,
monosodium phosphate buffer, pH 4.8,
no. 6346,
acetone, no. 14,
methanol, no. 6007,
E. Merck,
D-6100 Darmstadt 1

2026046918

glucose-6-phosphate dehydrogenase
(G6PDH, EC 1.1.1.49), no. 197726,
glucose-6-phosphate (G6P), no. 127027,
NADP, no. 128031,
NAD, no. 127981,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31

benzo(a)pyrene (B(a)P), no. 3176,
quinine sulfate (standard solution:
1.1 µg/ml 0.05 mol/l sulfuric acid),
no. 2-8838,
Carl Roth GmbH,
D-7500 Karlsruhe 21

bovine serum albumin (BSA), no.
A 4378,
tris(hydroxymethyl)aminomethane
(Tris), no. T 1503,
Sigma Chemie GmbH,
D-8021 Taufkirchen

9-hydroxybenzo(a)pyrene, no. 106,
3-hydroxybenzo(a)pyrene, no. 75,
IIT Research Institute,
Chicago Illinois, 60616, USA

Procedure

Enzyme incubation:

according to Van Cantfort, J.,
De Graeve, J. and Gielen, J.E.,
Biochem. Biophys. Res. Commun. 79:
505-512 (1977)

incubation time: 10 min

final concentrations of components
in assay mixture:

S9 fraction protein	12.00 mg/l
B(a)P	0.08 mmol/l
NAD	0.43 mmol/l
NADP	0.37 mmol/l
G6P	2.50 mmol/l
BSA	0.80 g/l
Tris-HCl, pH 7.6	50.00 mmol/l
magnesium chloride	5.00 mmol/l
G6PDH	1 U/ml

total assay volume: 0.50 ml

triplicate determination per sample

2026048919

HPLC separation

Injection volume: 20 µl

Column: RP-18, particle diameter: 10 µm,
column length: 250 mm, inner diameter: 4.6 mm

Oven and solvent temperature: 40 degrees centigrade

Solvent A: 500 ml/l acetonitrile
500 ml/l 10 mmol/l sodium dihydrogen phosphate buffer, pH 4.8

Solvent B: 1000 ml/l acetonitrile

Gradient: linear increase from 800 ml/l A and
200 ml/l B to 100 ml/l A and 900 ml/l B within 20 min

resulting gradient:
600 to 950 ml/l acetonitrile

Flow rate: 0.8 ml/min

Column pressure: 70 to 100 bar

Detection: fluorometric,
excitation wavelength: 305 nm,
emission wavelength: 430 nm,
slit of excitation and emission
monochromators: 15 nm

Calculations: standard curve: see FIGURE E

Scientific version: 4.Feb.81

Text version: 10.Aug.81

4.4.3 Determination of bacterial contamination of S9 fraction

Principle: determination of bacterial contamination by growth on nutrient agar plates

detection limited to aerobic cocci and rods

Time: immediately after preparation of

2026048920

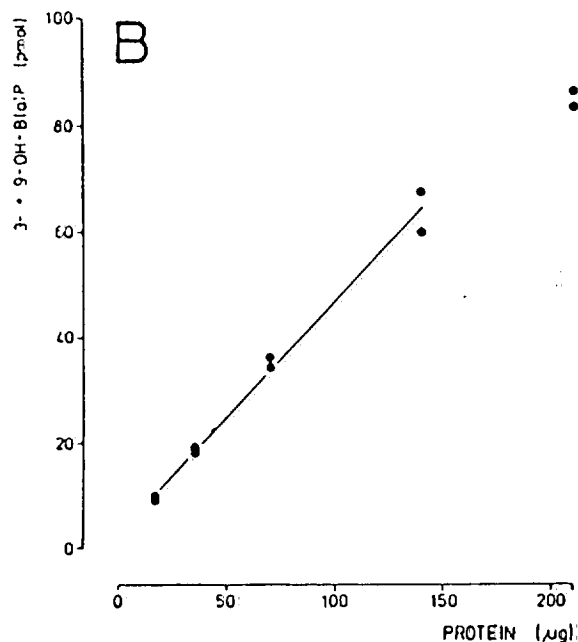
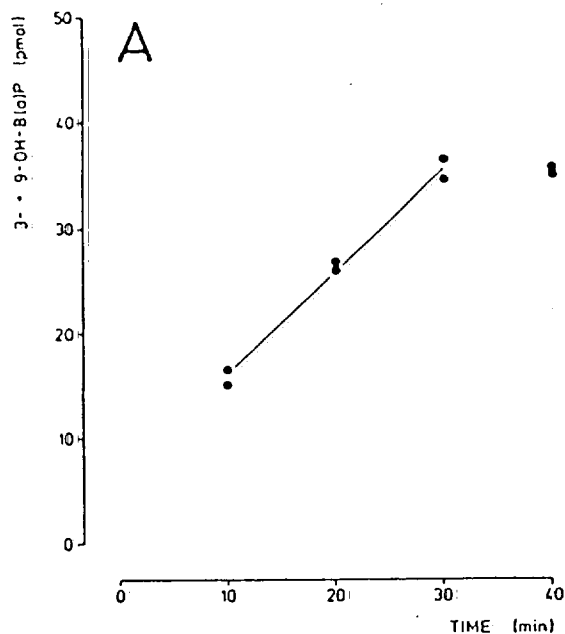


FIGURE E

DETERMINATION OF AHM ACTIVITY

Remarks: A: time response, 70 ug protein (a)

B: protein response (a), 30 min incubation time

date of determination: 19.Feb.81

scientific version: 4.Feb.81

(a) 9.8×10^5 m/s² microsomal pellet after dermal application
of 100 ul acetone/mouse

Source: <https://www.industrydocuments.ucsf.edu/docs/gsmm0000>

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Sample material and quantity: liver S9 fraction, 0.1 ml/plate

Results expressed in: CFU/ml

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

petri dishes: no. 1029,
100 mm x 15 mm, polystyrole,
sterilized,
Falcon,
Oxhard, CA. 93030, USA

colony counter: Colony Star 2,
Funke-Gerber,
D-1000 Berlin

Chemicals and reagents: nutrient agar, standard 1, no. 7881,
E. Merck,
D-6100 Darmstadt 1

Procedure: incubation of 2 inoculated plates at
37 degrees centigrade

manual counting of colonies after
3 d of incubation

Scientific version: 26.Jul.79
Text version: 21.Sep.81

4.4.4 Determination of promutagen activation

Principle: plate incorporation mutagenicity
assay with standard mutagens in
the presence of various doses
of S9 protein

2026048922

Sample material and quantity: S9 fraction 81.A, approx. 0.4 to 5 mg protein/plate

Results expressed in: revertants/plate

Equipment: see 4.6 Plate Incorporation Mutagenicity Assay

Chemicals and reagents:

standard mutagens:
benzo(a)pyrene, no. 3176,
Carl Roth GmbH,
D-7500 Karlsruhe

2-aminoanthracene (2-AA), no. A3,880-o,
Aldrich Europe,
D-4054 Nettetal 2

2-aminofluorene (2-AF), no. 820573,
Merck-Schuchardt,
D-8011 Hohenbrunn

WSC-I of 2R1, batch no. 49,
2.5 mg dry condensate/ml DMSO

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

concentrations of S9 mix components
prior to filtration and dilution:

MgCl ₂ 6-hydrate	16.0 mmol/l
KCl	66.6 mmol/l
sodium phosphate buffer (pH 7.4)	100.0 mmol/l
glucose-6-phosphate-Na ₂	5.0 mmol/l
NADP-Na ₂ 3-hydrate	4.0 mmol/l
S9 protein	10.0 g/l

top agar and minimal-glucose agar: see
4.6 Plate Incorporation Mutagenicity
Assay

Salmonella typhimurium, tester strains
see 4.5.1 Nutrient broth suspension
culture

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Procedure:

filtration of S9 mix prior to dilution, dilution with sodium phosphate buffer, 0.1 mol/l, pH 7.4

plating of S9 mixes in plate incorporation mutagenicity assay with standard mutagens, 4 plates per S9 mix dose and standard mutagens

doses of standard mutagens:

WSC-I 0.1 mg/plate

2-AA 2 ug/plate

2-AF' 2 ug/plate

B(a)P 5 ug/plate

incubation and counting of revertants:
see 4.6 Plate Incorporation Mutagenicity Assay

Scientific version:

26. Jun. 80

Text version:

10.Aug.82

4.5 Bacterial Tester Strains

4.5.1 Nutrient broth suspension culture

Principle:

overnight cultivation of tester
strain bacteria in complete nu-
trient broth

Time:

12 h prior to harvest

Sample material and quantity:

Salmonella typhimurium, tester strains TA 98 and TA 100 (a), frozen stock cultures

Equipment :

incubator shaker: model G 24,
New Brunswick Scientific,
Edison, New Jersey, USA

(a) kindly provided by Prof. Dr. Bruce Ames, University of California, Berkeley CA., USA

culture flask: Erlenmeyer flask,
100 ml, with long neck, used with
stainless steel caps,
Schott,
D-6500 Mainz

cryo tubes with screw caps, 2.0 ml,
no. 363401,
Nunc GmbH,
D-6200 Wiesbaden

Chemicals and reagents:

Difco-Bacto nutrient broth,
no. 0003-01,
Difco Laboratories,
Detroit, Michigan 48201, USA

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

Procedure

Preparation and growth:

inoculation of 30 ml nutrient broth
in culture flask with 10 µl of the
thawed and 10-fold diluted stock cul-
ture, cultures grown in a shaking
incubator at 37 degrees centigrade
with 200 rpm for 12 h

Storage of frozen stock
culture:

aliquots (0.1 ml) of tester strain
suspensions in nutrient broth with
87.5 ml DMSO/l at minus 196 degrees
centigrade in liquid nitrogen

Scientific version:

12.Jan.81

Text version:

29.Sep.81

2026048925

4.5.2 Determination of the density of bacteria suspension culture

Principle: photometric determination of the amount of light scattered by the suspension of bacteria

Time: at the beginning and at the end of the plate incorporation assay

Sample material and quantity: tester strain suspension culture, 1.0 ml

Results expressed in: absorbance unit (A)

Equipment: photometer: Spectronic 20, colorimeter test tubes, Bausch und Lomb, D-8043 Unterföhring

Chemicals and reagents: NaCl, no. 6400, E. Merck, D-6100 Darmstadt 1

Procedure: dilution:
1.0 ml suspension culture + 4.0 ml NaCl (9 g/l)

photometric determination:
wavelength: 565 nm
blank: NaCl (9 g NaCl/l)

absorbance calculation for the undiluted culture

Scientific version: 9.May 80
Text version: 21.Sep.81

2026048926

4.5.3 Determination of the number of viable bacteria

Principle: spreading of bacteria with top agar plating technique and counting of colony forming units

Time: immediately prior to start and at the end of each plate incorporation assay

Sample material and quantity: tester strain suspension culture, approx. 0.1 ml

Results expressed in: CFU/ml

Equipment:

- colony counter: Artek counter, model 880, Artek System, via Fisher Scientific, D-8000 München
- incubator: model 3916, Forma Scientific, Marietta, Ohio, USA
- petri dishes: no. 1029, 100 mm x 15 mm, polystyrole, sterilized, Falcon, Oxhard, CA. 93030, USA

Chemicals and reagents:

- minimal-glucose agar and top agar, composition: see 4.6 Plate Incorporation Mutagenicity Assay
- sodium chloride, no. 6400, E. Merck, D-6100 Darmstadt 1

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L-histidine hydrochloride 1-hydrate,
no. H 8125,
biotin, no. B 4501,
Sigma Chemie GmbH,
D-8021 Taufkirchen

histidine/biotin solution:
19.17 g histidine hydrochloride 1-hy-
drate and 24.4 mg biotin dissolved in
H₂O, sterilized by filtration

Procedure:

aliquots of tester strain suspen-
sion culture diluted 10⁶-fold in
9 g/l NaCl. 0.1 ml of this dilution
mixed with 2.0 ml top agar and 0.1 ml
histidine/biotin solution and plated
on minimal-glucose agar plates

incubation:
48 h at 37 degrees centigrade

counting:
manual and/or automatic counting
of colony forming units

Scientific version:
Text version:

13.Jan.81
8.Jul.82

4.5.4 Analyses of tester strain properties

Principle:

tester strain checked for:

- (1) auxotrophy in the form of
histidine requirement
- (2) absence of lipopolysaccharide
barrier in the form of sensi-
tivity to crystal violet
- (3) presence of R factor in the
form of resistance to ampicillin
- (4) lack of excision repair system
in the form of sensitivity to
UV light

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Time: at the beginning and at the end of each study

Sample material and quantity: tester strain suspension culture, approx. 0.5 ml

Equipment: UV source: Astralux F, no. 15136, 890 W, Astralux-Werke, Vienna, Austria

filter paper disks: no. 2668, diameter: 6 mm, Schleicher und Schüll, D-3354 Dassel

petri dishes: no. 1029, polystyrole, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1

incubator: model 3916, Forma Scientific, via Labotect, D-3400 Göttingen

Chemicals and reagents: L-histidine hydrochloride 1-hydrate, no. H 8125, biotin, no. B 4051, Sigma Chemie GmbH, D-8021 Taufkirchen

crystal violet, no. 1407, nutrient agar, standard 1, no. 7881, E. Merck, D-6100 Darmstadt 1

ampicillin sensitivity disk, 10 ug, Oxoid Deutschland GmbH, D-4230 Wesel

minimal-glucose agar and top agar, composition: see 4.6 Plate Incorporation Mutagenicity Assay

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Procedure of test for

Histidine requirement:

tester strain bacteria streaked on minimal-glucose agar plate without and with 0.1 ml of histidine and biotin solution (0.1 mol L-histidine hydrochloride 1-hydrate and 0.5 mmol biotin/l)

incubation at 37 degrees centigrade for 18 to 24 h

plates checked for growth

Crystal violet sensitivity:

10 ul crystal violet solution (1 g/l) applied to filter paper disk, placed onto complete nutrient agar plate and overlaid with 0.1 ml tester strain suspension culture

incubation at 37 degrees centigrade for 12 to 16 h

plate checked for inhibition zone

Ampicillin resistance:

ampicillin disk (10 ug) applied onto complete nutrient agar with tester strain bacteria plated

incubation at 37 degrees centigrade for 18 to 24 h

plate checked for growth around ampicillin disk

UV sensitivity:

tester strain bacteria to be tested streaked across nutrient agar plates and half of the streak irradiated for 30 s with a UV source at a distance of 10 cm

incubation at 37 degrees centigrade for 18 to 24 h

plate checked for growth inhibition

Scientific version:

20.May 81

Text version:

4.Aug.82

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4.6 Plate Incorporation Mutagenicity Assay

Principle: counting of revertants after growth in the presence of test substance

Time of top agar plating: see FIGURES A and B

Sample material and quantity:

- (1) 2-AA and 2-AF, 0.05 g/l, 2 ug/plate
- (2) test substance: WSC-I of test cigarette types PRO-3 and PRO-4
- (3) reference substance: standard reference cigarette WSC-I

Results expressed in: revertants/plate

Equipment:

- sterile filter: Millex SLHA, no. 0250 S and HAWG, no. 04700, 0.45 um pore size, Millipore S.A., F-6700 Molsheim, France
- incubator: model 3916, Forma Scientific, Marietta, Ohio, USA
- whirlmix: no. 34524-200, Cenco Instrumenten, Breda, The Netherlands
- thermostat: aluminium bloc thermostat, no. 2092, Gebr. Liebig, D-4800 Bielefeld
- test tubes: no. 3033, 16 mm x 125 mm, polystyrole, sterile, Falcon, Oxhard, CA. 93030, USA
- colony counter (manual): Colony Star 2, Funke-Gerber, D-1000 Berlin

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colony counter (automatic): Artek
counter, model 880,
Artek System,
Farmingdale, N.Y., USA

automatic pipettes:

- (1) Cornwall refilling syringes,
volume: 2 ml (for top agar),
E. Schütt,
D-3400 Göttingen
- (2) bottle-top dispenser "dis-
pensette", volume: 2 ml (for
S9 mix),
R. Brand GmbH + Co.,
D-6980 Wertheim
- (3) bottle-top dispenser "dis-
trivar", volume: 0.5 ml (for
bacteria suspension),
Gilson France, S.A,
F-95400 Villiers le Bel
- (4) adjustable pipettes: P 20, P 200
and P 1000, volume: 0.02, 0.2
and 1 ml (for test substances),
Gilson France, S.A,
F-95400 Villiers le Bel

Chemicals and reagents:

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

2-aminoanthracene (2-AA), no. A 3,880-0,
Aldrich Europe,
D-4054 Nettetal 2

2-aminofluorene (2-AF), no. 820573,
Merck-Schuchardt,
D-8011 Hohenbrunn

composition of minimal-glucose agar,
top agar and S9 mix: see TABLES E,
F and G

Salmonella typhimurium tester strains
see 4.5.1 Nutrient broth suspension
culture

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COMPOUND	ART. NO.	SOURCE	CONCENTRATION (g/l)
agar	0140-01	Difco (a)	15.0
glucose 1-hydrate	8342	Merck (b)	20.0
MgSO ₄ 7-hydrate	5886	Merck (b)	0.2
K ₂ HPO ₄ 3-hydrate	5099	Merck (b)	13.1
citric acid 1-hydrate	244	Merck (b)	2.0
NaNH ₄ HPO ₄ 4-hydrate	6682	Merck (b)	3.5

TABLE E

COMPOSITION OF MINIMAL-GLUCOSE AGAR

preparation:

salts and glucose as 10-fold concentrated solutions separately

sterilization:

1 bar, 121 degrees centigrade, 15 min, except glucose, which was added to the molten medium as a sterile-filtered solution

filling into petri dishes (c):

automatically, approx. 30 ml molten agar/plate. Drying: excess water on the solid agar plates removed by exposure of the covered plates at 37 degrees centigrade for 3 d

storage of minimal-glucose agar plates:
after drying at RT

(a) Difco Laboratories, Detroit, Michigan, USA

(b) E. Merck, D-6100 Darmstadt 1

(c) automatic petri dish filler, Laboramat II,
Labora, D-6395 Weilrod-Winden

COMPOUND	ART. NO.	SOURCE	CONCENTRATION (g/l)
agar	0140-01	Difco (a)	0.55
sodium chloride	6400	Merck (b)	0.45
L-histidine hydrochloride 1-hydrate	H 8125	Sigma (c)	0.0087
biotin	B 4501	Sigma (c)	0.011

TABLE F

COMPOSITION OF TOP AGAR

preparation:

histidine and biotin prepared as a 10-fold concentrated solution

sterilization:

- (1) histidine/biotin solution: sterile filtration
- (2) agar/sodium chloride: 1 bar, 121 degrees centigrade, 15 min

storage:

- (1) histidine/biotin at 4 degrees centigrade
- (2) agar/sodium chloride at RT

prior to use:

agar/sodium chloride remolten by boiling in a water bath for approx. 20 min, addition of histidine/biotin after cooling down to approx. 45 degrees centigrade

(a) Difco Laboratories, Detroit, Michigan, USA

(b) E. Merck, D-6100 Darmstadt 1

(c) Sigma Chemie GmbH, D-8021 Taufkirchen

COMPOUND	ART. NO.	SOURCE	CONCENTRATION
MgCl ₂ 6-hydrate	5833	Merck (a)	6.4 mmol/l
KCl	4936	Merck (a)	26.4 mmol/l
sodium phosphate buffer, pH 7.4	6346 and 6580	Merck (a)	100 mmol/l
glucose-6- phosphate-Na ₂	127027	Boehringer (b)	2.3 mmol/l
NADP-Na ₂ 3-hydrate	128058	Boehringer (b)	1.6 mmol/l
S9 protein	-	-	4.0 g/l (c)

TABLE G

COMPOSITION OF S9 MIX

sterilization:

magnesium chloride/potassium chloride and sodium phosphate buffer
are sterilized separately: 1.02 x 10⁵ Pa, 121 degrees centigrade,
15 min

glucose-6-phosphate and NADP: filter-sterilized (pore size:
0.45 µm)

storage:

magnesium chloride/potassium chloride and sodium phosphate buffer:
at 4 degrees centigrade

glucose-6-phosphate and NADP at minus 80 degrees centigrade

prior to use:

sterile filtration of the complete S9 mix

(a) E. Merck, D-6100 Darmstadt 1

(b) Boehringer Mannheim GmbH, D-6800 Mannheim 31

(c) concentration of S9 protein prior to filtration

Procedure

Plating mixture
preparation:components added in the following
order:

- (1) 2.0 ml top agar, 45 degrees centigrade
- (2) test substance, 2 plates/dose,
- (3) 0.1 ml tester strain suspension culture, containing approx. 10^8 viable bacteria at 0 degrees centigrade
- (4) 0.5 ml S9 mix or S9 mix buffer at 0 degrees centigrade

Top agar plating:

components mixed by rotating the test tube gently on a whirlmix, then poured on minimal-glucose agar plates and spread evenly on the surface by wobbling. The mixing, pouring and spreading of the top agar occurred within 20 s. The plates allowed to harden for 3 to 6 min and then transferred to the dark incubator

Incubation:

48 h at 37 degrees centigrade in the dark

Counting of revertants:

if determination of the number of revertants not performed immediately at the end of the incubation period, plates stored at 4 degrees centigrade for not longer than 24 h

if necessary, plates brought to RT and the number of revertants counted manually and/or automatically

automatic counting:

each plate counted 3 times, rotation of the plate for 120 degrees between each count, highest count used for the calculation of revertants

Scientific version:
Text version:

3.Jul.80
30.Sep.81

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4.7 Spot Assay

Principle: counting of revertants after growth in the presence of diagnostic mutagens applied to filter paper disk

Time: at the day of plate incorporation mutagenicity assay

Sample material and quantity: (1) 9-aminoacridine, 0.5 g/l ethanol, 10 ug/plate
(2) MMS, 2 ul/plate
(3) MNNG, 0.1 g/l DMSO, 2 ug/plate

Results expressed in: revertants/plate

Equipment: incubator: model 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingen

colony counter (automatic): Artek
counter, model 880,
Artek System,
via Fisher Scientific,
D-8000 München

filter paper disks: no. 2668, diameter: 6 mm,
Becton Dickinson GmbH,
D-6900 Heidelberg

Chemicals and reagents: dimethyl sulfoxide (DMSO),
for spectroscopy, no. 2950,
ethanol, no. 972,
E. Merck,
D-6100 Darmstadt 1

9-aminoacridine, no. 3,840-1,
N-Methyl-N'-nitro-N-nitrosoguanidine
(MNNG), no. 12,994-1,
Ega Chemie,
D-7924 Steinheim

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methyl methanesulfonate (MMS),
no. 12,992-5,
Aldrich Europe,
D-4054 Nettetal 2

Salmonella typhimurium tester strains:
see 4.5.1 Nutrient broth suspension
culture

top agar and minimal-glucose agar,
composition: see 4.6 Plate Incorpora-
tion Mutagenicity Assay

Procedure:

top agar plating of tester strains on
minimal-glucose agar

diagnostic mutagens applied to filter
paper disks placed on top of hardened
agar plates

incubation:
48 h at 37 degrees centigrade in the
dark

automatic counting of revertants:
see 4.6 Plate Incorporation Muta-
genicity Assay

Scientific version:
Text version:

3.Jul.79
3.Jun.82

4.8 Reversion Assay

Principle:

analysis of growth of individual
colonies on minimal-glucose agar
without histidine

Time:

2 d after start of plate in-
corporation assay

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Sample material and quantity: 10 individual colonies from 1 mutagenicity assay plate with the highest dose of test substance

Results expressed in: number of colonies grown (histidine prototroph/10 colonies inoculated)

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

Chemicals and reagents: minimal-glucose agar, composition:
see 4.6 Plate Incorporation Mutagenicity Assay

Procedure: streaking of revertant colonies on
minimal-glucose agar plate with a
platinum loop, incubation of plates
at 37 degrees centigrade for 48 h
and counting of colonies grown
after incubation

Scientific version: 25.Mar.80
Text version: 30.Sep.81

4.9 Statistical Evaluation

Primary data (revertants/
plate): calculation of MEAN and RSD from
all plates for each cigarette,
also calculated separately for
assay 1 and assay 2, data not
corrected for automatically counting

Specific mutagenicity: equivalent to regression coefficient
"a" of a linear dose-response curve
 $y = ax + b$

2-AF: increase of revertants per μg 2-AF

WSC-I: increase of revertants per mg dry
condensate

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Relative specific
mutagenicity:

mutagenicity of WSC-I of cigarettes
related to the mutagenicity of the
standard reference cigarette 2R1
prepared and assayed in the same assay
(internal standard set to 100 percent)

Relative difference:

absolute difference between 2 values
(A and B) divided by the mean of them:

$$\frac{|A - B|}{(A + B)/2}$$

Statistical significance of
the difference between the
specific mutagenicities of
2 independent assays:

statistical significance reached at
the level of significance equal to
0.017 with a relative difference of
the specific mutagenicities by
25 percent (a)

Statistical significance of
the difference between the
specific mutagenicities of
2 individual cigarettes:

statistical significance reached at
the level of significance equal to
0.05 with a relative difference of
the specific mutagenicities by
16 percent (a)

Scientific version:
Text version:

21.Jul.81
25.Aug.82

- (a) In a basic biometric study with tester strain TA 98 a single cigarette type was assayed according to the INBIFO WSC-I standard mutagenicity assay procedure (same procedure as in the present study: 2 independent assays, 4 doses and 64 plates/cigarette type). For the lack of basic biometric study with tester strain TA 100, the limit of the relative difference for 2 cigarettes or 2 assays is set to 0.16 or 0.25 respectively following the biometric study with TA 98.

5 STORAGE OF MATERIALS AND RECORDS

=====

Test substance

Test cigarettes:

no storage of test cigarettes

Condensates:

5 ml of each condensate preparation transferred at day of mutagenicity assay into plastic tube with screw cap and stored at minus 80 degrees centigrade for at least 1 year

approx. 15 ml of each condensate preparation stored at plus 4 degrees centigrade for approx. 1 month

Proposal, records and evaluation sheets:

stored in our archives for at least 5 years, they can be claimed by the client

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6 RESULTS AND DISCUSSION =====

6.1 Text

6.1.1 WSC-I of cigarettes

- 6.1.1.1 Dry condensate, nicotine and water concentration and puff number
(see TABLES 1 and 2)

Whole smoke condensates (WSC-I) were prepared 7 or 8 days prior to the mutagenicity assays (FIGURES A and B) using a glass impaction trap (FIGURE C). On each day of cigarette condensate preparation, condensates of the 2R1 standard reference cigarette were also prepared. The dry condensate nicotine and water concentration of WSC-I/DMSO suspension of the 2R1 standard reference cigarette were in the expected range.

- 6.1.1.2 Bacteriological examination of WSC-I
(see TABLE 3)

The diluted condensate suspensions were found to be free from bacteria growing on minimal-glucose agar.

6.1.2 Properties and responses of the tester strains

- 6.1.2.1 Properties
(see TABLE 4)

Cultures from frozen stock cultures of *Salmonella typhimurium* tester strains TA 98 and TA 100 were used for mutagenicity testing in this study. With tester strain TA 98 mutagens causing frame-

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shift mutation and with TA 100 mutagens causing base-pair substitution can be detected. The strains were checked for properties essential to the plate incorporation mutagenicity assay as recommended in the basic "method paper" by Ames et al. (1975):

- (1) auxotrophy in the form of histidine requirement,
- (2) the absence of the lipopolysaccharide barrier in the form of sensitivity to crystal violet,
- (3) the presence of the ampicillin resistance carrying extrachromosomal R factor and
- (4) the lack of an excision repair system (uvrB) in the form of sensitivity to UV light.

The bacteria responded in a manner characteristic for tester strains TA 98 and TA 100 as described by Ames et al. (1975).

6.1.2.2 Spontaneous reversion

(see TABLES 5 to 8 and FIGURES 1 and 2)

In order to determine the spontaneous reversion of the tester strains in the absence of cigarette smoke condensates, the solvent DMSO used for the condensates was mixed with top agar, tester strain bacteria and either S9 mix or S9 mix buffer and plated on minimal-glucose agar. The number of spontaneous revertants was determined automatically. Determinations were performed at the beginning and at the end of the mutagenicity assay on each day.

The number of spontaneous TA 98 revertants per plate was found to be 23.8 ± 1.4 (a) in the absence of S9 protein, when approx. 1.0×10^8 viable bacteria were added to each plate.

(a) MEAN \pm SE, N = 16

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The number of spontaneous TA 100 revertants per plate was found to be 121.3 ± 1.7 (a) in the absence of S9 protein, when approx. 1.0×10^8 viable bacteria were added to each plate.

The results of the spontaneous reversion are in agreement with our experience with the strains and findings by Ames et al. (1975) as well as by de Serres and Shelby (1979).

6.1.2.3 Mutagenicity of diagnostic mutagens (see TABLES 9 to 12 and FIGURES 3 and 4)

Positive and negative mutagenesis controls were included in this study using 5 diagnostic mutagens to confirm the reversion properties of the tester strains and the metabolic activity of the S9 protein used. The results of MMS, MNNG and 9-aminoacridine spot assays were in accordance with the results expected and published by Ames et al. (1975).

2-Aminoanthracene (2-AA) and 2-aminofluorene (2-AF) were tested in the plate incorporation mutagenicity assay at a single dose in the presence of S9 protein. The number of revertants per plate was nearly the same in both assays. These results are in accordance with those published by Zeiger et al. (1979) and Simmon (1979) and with those expected for TA 98 and TA 100.

In addition, an almost linear dose-response curve was obtained with 2-AF between 0 and 3 micrograms per plate in 2 plate incorporation mutagenicity assays. The mean specific mutagenicity was 124.5 revertants per microgram 2-AF with TA 98 and 48.5 with TA 100. These results were in agreement with previous studies.

(a) MEAN \pm SE, N = 16

6.1.3 Properties of S9 protein

(see TABLES 13 to 15 and FIGURE 5)

The S9 protein was prepared from liver homogenate of Aroclor 1254-induced rats. Analytical data of the preparation used in this study are shown in TABLE 15. The specific AHM activity was 121.6 U/mg protein in the expected range.

The activity of the S9 protein used in this study for the bio-transformation of promutagens to mutagens in the plate incorporation assay was determined with B(a)P, WSC-I of cigarette 2R1 and 2 diagnostic mutagens. The amount of 0.9 to 1.9 milligrams S9 protein per plate was found to be suitable for the plate incorporation mutagenicity assay of WSC-I using tester strains TA 98. The response of tester strain TA 98 to B(a)P and WSC-I of cigarette 2R1 metabolically activated by the S9 protein was in the expected range.

During the preparation of S9 mix, the S9 protein was diluted to 4 g/l and the S9 mix was sterile-filtered. During this filtration, the protein concentration was reduced to approx. 3.1 g/l S9 mix. The final amount of S9 protein per mutagenicity assay plate was 1.6 milligrams.

6.1.4 Mutagenicity of cigarette smoke condensates

6.1.4.1 Experimental approach

(see TABLES 16 to 29 and FIGURES A and B)

WSC-I of cigarettes of project "LEAR" and 2R1 were assayed for their mutagenicity in the plate incorporation assay, using Salmonella typhimurium tester strains TA 98 and TA 100 with metabolic activation by S9 protein from Aroclor 1254-induced rat livers.

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Condensates of each cigarette were assayed in 2 independent assays on 2 different days. All WSC-I were diluted to a final concentration of 2.5 milligrams dry condensate per milliliter and were assayed in the presence of S9 protein at 4 different condensate doses using 2 plates per dose. The number of revertants were counted automatically.

6.1.4.2 Dose response

(see FIGURES 6 to 19)

All condensates were assayed at 4 doses: 0, 0.05, 0.10 and 0.15 milligrams dry condensate/plate. Depending on the dose an approx. linear increase in the number of revertants was observed with condensates of all cigarettes.

6.1.4.3 Reversion assay

(see TABLES 30 and 31)

In order to test, whether colonies grown on minimal-glucose agar plates with limited histidine supply are revertants (a), individual colonies after 2-day growth on plates, to which the high dose of condensates had been added, were tested for histidine prototrophy on agar plates without histidine.

140 out of 140 (100 percent) colonies of tester strain TA 98 and 140 out of 140 (100 percent) colonies of tester strain TA 100 from mutagenicity test plates were found to be histidine prototrophs (revertants) in the subsequent reversion assay on plates without histidine.

- (a) A trace amount of histidine was added to the top agar in the plate incorporation assay to allow the bacteria on the plate to undergo several divisions which are in many cases necessary for mutagenesis to occur. In case of massive cell death during exposure of the bacteria to a test substance more histidine is available to the individual surviving bacteria, and they undergo more cell divisions forming small colonies ("false revertants") which can be mistaken for revertants (Ames et al. (1975)).

6.1.4.4 Specific mutagenicity

(see TABLES 32 to 51, FIGURES 2o and 21)

The mutagenic activity was expressed as the specific mutagenicity, defined as the regression coefficient "a" of the linear dose-response curve $y = ax + b$. The specific mutagenicity was calculated as the extrapolated increase in the number of revertants per milligram of dry condensate.

Using tester strain TA 98 to detect frameshift mutations, the specific mutagenicity of each cigarette obtained from assay 1 was statistically not different to that of assay 2. The specific mutagenicity was 2235 revertants per milligram dry condensate for LEAR o-17-2, 35o8 for LEAR o-17-3, 193o for LEAR 2-17-2, 2942 for LEAR 2-17-3, 2o23 for LEAR 4-17-2, 2988 for LEAR 4-17-3 and 1536 for 2R1. The cigarettes with the last digit number "2" were found to be statistically significantly less mutagenic than the corresponding cigarettes with the last digit number "3" (a). The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

Using tester strain TA 1oo to detect base-pair substitution, the specific mutagenicity obtained in assay 1 was statistically not different to that in assay 2 for each cigarette. The specific mutagenicity was 859 revertants per milligram dry condensate for LEAR o-17-2, 1354 for LEAR o-17-3, 897 for LEAR 2-17-2, 1185 for LEAR 2-17-3, 863 for LEAR 4-17-2, 1ooo for LEAR 4-17-3 and 696 for 2R1. The cigarettes LEAR o-17-2 and LEAR 2-17-2 were found to be statistically significantly less mutagenic, whereas the cigarette LEAR 4-17-2 was only numerically less mutagenic than the corresponding cigarettes with the last digit number "3" (a). The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

(a) LEAR cigarettes with the last digit number "2" differed from the corresponding cigarettes in higher amount of reducing sugars of filler (see TABLE B, PAGE 3-4).

6.1.4.5 Relative specific mutagenicity (see TABLES 48 and 49, FIGURE 22)

In order to compensate variations from study to study in the condensate preparation and mutagenicity assay performance, condensates of the standard reference cigarette 2R1 were used as an internal standard. These standard condensates were prepared on the same day and stored and assayed under the same conditions as the other cigarette condensates. The specific mutagenicity of the cigarettes were compared to that of the standard cigarette 2R1 which was set to 100 percent.

In case of tester strain ta 98 the relative specific mutagenicity was 146 percent for LEAR o-17-2, 228 for LEAR o-17-3, 126 for LEAR 2-17-2, 192 for LEAR 2-17-3, 132 for LEAR 4-17-2 and 195 for LEAR 4-17-3.

In case of tester strain TA 100 the relative specific mutagenicity was 123 percent for LEAR o-17-2, 195 for LEAR o-17-3, 129 for LEAR 2-17-2, 170 for LEAR 2-17-3, 124 for LEAR 4-17-2 and 144 for LEAR 4-17-3.

6.1.5 Conclusion

The specific mutagenicities of the condensates tested show a range from the highest value of LEAR o-17-3 to the lowest value of 2R1 with a factor of 2.3 for frameshift mutation and 1.9 for base-pair substitution.

For frameshift mutation as well as for base-pair substitution the condensates of cigarettes with the last digit number "2" are less mutagenic than the corresponding cigarettes with the last digit number "3". The condensates of all cigarettes of project "LEAR" are more mutagenic than that of standard reference cigarette 2R1.

6.2 Tables and Figures

CIGARETTE	DATE OF CON- DENSATE PRE- PARATION	BATCH NO.	DRY CON- DENSATE CONC. (g/l)	NICOTINE CONC. (g/l)	WATER CONC. (g/l)	NUMBER OF PUFFS (1/cig.)
2R1	2.Nov.81	54	45.70	3.37	6.90	11.3
		55	46.99	3.44	6.25	11.1
	17.Nov.81	56	50.82	3.85	6.88	11.5
		57	47.33	3.65	5.52	11.2
LEAR o-17-2	2.Nov.81	o1	19.67	1.62	5.29	8.1
		o2	20.00	1.63	5.02	7.2
	17.Nov.81	o3	15.88	1.49	2.37	8.7
		o4	15.99	1.55	2.24	8.5
LEAR o-17-3	2.Nov.81	o1	15.69	1.95	2.94	8.0
		o2	17.19	2.24	4.52	8.7
	17.Nov.81	o3	16.51	2.08	2.82	8.8
		o4	16.59	2.07	2.50	8.8
LEAR 2-17-2	2.Nov.81	o1	19.42	1.43	4.09	8.8
		o2	17.64	1.46	4.95	9.0
	17.Nov.81	o3	17.47	1.41	2.89	8.2
		o4	16.31	1.31	1.85	8.4
LEAR 2-17-3	2.Nov.81	o1	19.73	1.60	4.40	9.1
		o2	12.44	1.21	3.28	9.0
	17.Nov.81	o3	19.64	1.51	2.11	8.9
		o4	16.95	1.45	1.59	9.0
LEAR 4-17-2	2.Nov.81	o1	17.36	1.50	3.53	9.0
		o2	19.18	1.66	5.26	8.8
	17.Nov.81	o3	18.34	1.50	2.18	8.7
		o4	18.72	1.64	2.53	8.4
LEAR 4-17-3	2.Nov.81	o1	18.31	1.83	4.64	8.2
		o2	17.75	1.91	4.27	8.2
	17.Nov.81	o3	18.44	1.88	2.01	8.5
		o4	17.86	1.86	1.79	8.5

TABLE 1

CONCENTRATION OF DRY CONDENSATE, NICOTINE AND WATER OF WSC-I/DMSO SUSPENSION
AND PUFF NUMBER OF CIGARETTES

2026048949

CIGARETTE	DRY CONDENSATE		NICOTINE		WATER		NUMBER OF	
	CONC.		CONC.		CONC.		PUFFS	
	(g/l)		(g/l)		(g/l)		(1/cig.)	
	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
2R1	55.50	0.65	3.48	0.11	6.39	0.33	11.3	0.1
LEAR 0-17-2	17.89	1.13	1.57	0.03	3.73	0.82	8.1	0.3
LEAR 0-17-3	16.50	0.31	2.09	0.06	3.20	0.45	8.6	0.2
LEAR 2-17-2	17.71	0.64	1.40	0.03	3.45	0.68	8.6	0.2
LEAR 2-17-3	17.19	1.71	1.44	0.08	2.85	0.63	9.00	0.0
LEAR 4-17-2	18.40	0.39	1.58	0.04	3.38	0.69	8.73	0.1
LEAR 4-17-3	18.09	0.17	1.87	0.02	3.18	0.74	8.35	0.1

TABLE 2

MEAN CONCENTRATION OF DRY CONDENSATE, NICOTINE AND WATER OF WSC-I/DMSO SUSPENSION AND MEAN PUFF NUMBER OF CIGARETTES, N = 4

2026048950

DATE	CIGARETTE	CONDENSATE BATCH NO.	BACTERIAL CONTAMINATION (CFU/o.15 mg dry cond.)	
			PLATE 1	2
9.Nov.81	2R1	54	o	o
	LEAR o-17-2	o1	o	o
	LEAR o-17-3	o1	o	o
	LEAR 2-17-2	o1	o	o
	LEAR 2-17-3	o1	o	o
	LEAR 4-17-2	o1	o	o
	LEAR 4-17-3	o1	o	o
10.Nov.81	2R1	54	o	o
	LEAR o-17-2	o1	o	o
	LEAR o-17-3	o1	o	o
	LEAR 2-17-2	o1	o	o
	LEAR 2-17-3	o1	o	o
	LEAR 4-17-2	o1	o	o
	LEAR 4-17-3	o1	o	o
24.Nov.81	2R1	56	o	o
	LEAR o-17-2	o3	o	o
	LEAR o-17-3	o3	o	o
	LEAR 2-17-2	o3	o	o
	LEAR 2-17-3	o3	o	o
	LEAR 4-17-2	o3	o	o
	LEAR 4-17-3	o3	o	o
25.Nov.81	2R1	56	o	o
	LEAR o-17-2	o3	o	o
	LEAR o-17-3	o3	o	o
	LEAR 2-17-2	o3	o	o
	LEAR 2-17-3	o3	o	o
	LEAR 4-17-2	o3	o	o
	LEAR 4-17-3	o3	o	o

TABLE 3

BACTERIOLOGICAL EXAMINATION OF WSC-I OF CIGARETTES

2026048951

PARAMETER	TESTER STRAIN	
	TA 98	TA 100
histidine requirement		
growth without histidine	o	o
growth with histidine	+	+
crystal violet sensitivity	+	+
ampicillin resistance	+	+
uv sensitivity	+	+

TABLE 4

PROPERTIES OF THE TESTER STRAINS TA 98 AND TA 100

dates of determinations: 6.Oct.81
30.Nov.81

2026048952

DATE	TEST NO.	ABSOR- BANCE	VIABILITY (CFU/plate)				NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (bacteria/plate)	
			PLATE				MEAN (10E6)	RSD
			1	2	3	4		
								(o/o)
9.Nov.81	1	1.78	158	105	126	166	138.8	20.5
	2	1.55	81	67	49	107	76.0	32.2
24.Nov.81	1	1.75	121	146	101	160	132.0	19.9
	2	1.60	49	99	42	80	67.5	39.6
9.Nov.81 and 24.Nov.81	1 and 2	-	-	-	-	-	103.6 ± 10.2 (a)	39.3

TABLE 5

ABSORBANCE, VIABILITY AND NUMBER OF VIABLE TA 98 BACTERIA PLATED IN THE
MUTAGENICITY ASSAY

After growth for 12 h, suspension cultures were diluted 10E6-fold and 0.1 ml of the dilution plated onto minimal-glucose agar plates with a sufficient amount of histidine (10 µmol/plate). Tests were performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

(a) MEAN ± SE, N = 16

DATE	TEST NO.	SPONTANEOUS REVERTANTS (number of revertants/ plate)				STATISTICAL PARAMETER	
		PLATE				MEAN	RSD
		1	2	3	4		
							(o/o)
9.Nov.81	1	24	22	22	30	24.5	15.5
	2	14	19	19	23	18.8	19.7
24.Nov.81	1	28	22	30	33	28.3	16.4
	2	27	14	25	28	23.5	27.5
9.Nov.81 and 24.Nov.81	1 and 2	-	-	-	-	23.8 \pm 1.4 (a)	23.2

TABLE 6

SPONTANEOUS REVERTANTS OF STRAIN TA 98 IN THE ABSENCE OF S9 PROTEIN

Test performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

(a) MEAN \pm SE, N = 16

DATE	TEST NO.	ABSORBANCE	VIABILITY (CFU/plate)				NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (bacteria/plate)	
			PLATE				MEAN (10E6)	RSD (o/o)
			1	2	3	4		
10.Nov.81	1	1.75	71	77	85	86	79.8	8.9
	2	1.60	50	41	80	-	57.0	35.8
25.Nov.81	1	1.70	123	120	140	144	131.8	9.1
	2	1.60	100	116	118	114	112.0	7.3
10.Nov.81 and 25.Nov.81	1 and 2	-	-	-	-	-	97.7 ± 8.0 (a)	31.6

TABLE 7

ABSORBANCE, VIABILITY AND NUMBER OF VIABLE TA 100 BACTERIA PLATED IN THE MUTAGENICITY ASSAY

After growth for 12 h, suspension cultures were diluted 10E6-fold and 0.1 ml of the dilution plated onto minimal-glucose agar plates with a sufficient amount of histidine (10 µmol/plate). Tests were performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

(a) MEAN ± SE, N = 16

DATE	TEST NO.	SPONTANEOUS REVERTANTS (number of revertants/ plate)				STATISTICAL PARAMETER	
		PLATE				MEAN	RSD
		1	2	3	4		
							(o/o)
10.Nov.81	1	131	121	124	120	124.0	4.0
	2	131	115	121	103	117.5	10.0
25.Nov.81	1	121	117	131	124	123.3	4.8
	2	117	120	120	125	120.5	2.8
10.Nov.81 and 25.Nov.81	1 and 2	-	-	-	-	121.3 \pm 1.7 (a)	5.7

TABLE 8

SPONTANEOUS REVERTANTS OF STRAIN TA 100 IN THE ABSENCE OF S9 PROTEIN

Test performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

 (a) MEAN \pm SE, N = 16

DATE	MUTAGENICITY (mean number of revertants/plate)				
	MMS	MNNG	9-AMINO- ACRIDINE	2-AA + S9 PROT. (a)	2-AF + S9 PROT. (a)
	DOSE (mg/plate)				
	2.8	0.002	0.010	0.002	0.002
9.Nov.81	2	9	0	1502	227
24.Nov.81	4	3	0	1439	250
SUMMARIZED RESPONSE	0	0	0	+++	++
PUBLISHED RESPONSE	0,+	0	0	+++	+++
REFERENCE	Ames et al. (1975)	Ames et al. (1975)	Ames et al. (1975)	Zeiger et al. (1979) (b)	Simmon (1979) (c)

TABLE 9

MUTAGENICITY OF DIAGNOSTIC MUTAGENS TOWARDS STRAIN TA 98

When not specified, tests were performed in the absence of S9 protein, the mean of 2 plates/diagnostic mutagen. 2-AA and 2-AF in the presence of S9 protein were dissolved in top agar, all other substances were tested in spot assays.

0: .LT.20 revertants/plate

+: 20 to 100 revertants/plate

++: 100 to 400 revertants/plate

+++: .GT.400 revertants/plate

+, 0: variable response, .LT.20 to 100 revertants/plate

Number of colonies was corrected for spontaneous revertants.

(a) 1.5 to 1.6 mg S9 protein/plate

(b) 2.35 mg S9 protein/plate

(c) 1 to 2 mg S9 protein/plate

DATE	MUTAGENICITY (mean number of revertants/plate)				
	MMS	MNNG	9-AMINO- ACRIDINE	2-AA + S9 PROT. (a)	2-AF + S9 PROT. (a)
	DOSE (mg/plate)				
	2.8	0.002	0.010	0.002	0.002
10.Nov.81	600	320	11	1780	88
24.Nov.81	536	338	0	1650	112
SUMMARIZED RESPONSE	+++	++	0	+++	++
PUBLISHED RESPONSE	+++	+++	0	-	+++
REFERENCE	Ames et al. (1975)	Ames et al. (1975)	Ames et al. (1975)	-	Simmon (1979) (b)

TABLE 10

MUTAGENICITY OF DIAGNOSTIC MUTAGENS TOWARDS STRAIN TA 100

When not specified, tests were performed in the absence of S9 protein, the mean of 2 plates/diagnostic mutagen. 2-AA and 2-AF in the presence of S9 protein were dissolved in top agar, all other substances were tested in spot assays.

0: .LT.20 revertants/plate

+: 20 to 100 revertants/plate

++: 100 to 400 revertants/plate

+++: .GT.400 revertants/plate

+, 0: variable response, .LT.20 to 100 revertants/plate

Number of colonies was corrected for spontaneous revertants.

(a) 1.5 to 1.6 mg S9 protein/plate

(b) 1 to 2 mg S9 protein/plate

DATE	DOSE	MUTAGENICITY (number of revertants/ plate)						REGRESS. COEFF.	CORR. COEFF.
		PLATE				MEAN	RSD		
		1	2	3	4				
	(ug/ plate)						(o/o)	(1/ug)	
9.Nov.81	0	35	29	37	40	35.3	13.2		
	1	135	106	114	92	111.8	16.1		
	2	222	271	269	288	262.5	10.8		
	3	387	355	403	369	378.5	5.5	118.0	0.993
24.Nov.81	0	32	31	30	23	29.0	14.1		
	1	117	123	154	116	127.5	14.1		
	2	271	254	331	261	279.3	12.6		
	3	417	397	443	404	415.3	4.9	131.1	0.997
9.Nov.81 and 24.Nov.81	-	-	-	-	-	-	-	124.5 (a)	0.992

TABLE 11

MUTAGENICITY OF 2-AMINOFLUORENE TOWARDS STRAIN TA 98
IN THE PRESENCE OF S9 PROTEIN

(a) MEAN

DATE	DOSE	MUTAGENICITY (number of revertants/ plate)				MEAN	RSD	REGRESS. COEFF.	CORR. COEFF.
		PLATE							
		1	2	3	4				
	(ug/ plate)						(o/o)	(1/ug)	
10.Nov.81	0	121	98	107	124	112.5	10.8		
	1	158	177	150	164	162.3	7.0		
	2	185	207	201	210	200.8	5.6		
	3	249	282	255	258	261.0	5.6	48.4	0.997
25.Nov.81	0	102	120	123	-	117.3	8.8		
	1	182	185	168	196	182.8	6.3		
	2	194	245	227	252	229.5	11.3		
	3	271	246	265	274	264.0	4.8	48.7	0.990
9.Nov.81 and 24.Nov.81	-	-	-	-	-	-	-	48.5 (a)	0.992

TABLE 12

MUTAGENICITY OF 2-AMINOFLUORENE TOWARDS STRAIN TA 100
IN THE PRESENCE OF S9 PROTEIN

(a) MEAN

PARAMETER	UNIT	S9 FRACTION
batch no.	-	81.A
number of rats	-	19
date of Aroclor administration	-	8.Aug.81
date of sacrifice	-	13.Aug.81
body weight	g	220.0
liver weight	g	10.3
date of S9 preparation	-	13.Aug.81
protein concentration	g/l	42.8
specific AHM activity	U/mg protein	121.6
bacterial contamination (a)	CFU/ml	18

TABLE 13

ANALYTICAL DATA OF S9 PROTEIN, BATCH NO. 81.A

(a) Results of unfiltered S9 fraction. S9 protein added to the other components of the S9 mix and sterile-filtered prior to use in the plate incorporation assay.

TEST SUBSTANCE	S9 PROTEIN (mg/plate)	MUTAGENICITY (number of revertants/ plate)				STATISTICAL PARAMETER	
		PLATE				MEAN	RSD
		1	2	3	4		
							(o/o)
DMSO	o	26	19	15	30	22.5	30.0
	0.46	24	18	27	28	24.3	18.6
	0.92	28	19	16	34	24.3	34.1
	1.85	20	22	20	26	22.0	12.9
	3.47	27	43	30	36	34.0	20.8
	4.62	33	29	35	36	33.3	9.3
2R1	o	17	23	26	24	22.5	17.2
	0.46	69	75	89	79	78.0	10.8
	0.92	231	165	181	206	195.8	14.8
	1.85	185	198	233	218	208.5	10.2
	3.47	73	163	155	179	142.5	33.3
	4.62	116	82	84	90	93.0	16.9
B(a)P	o	26	21	28	25	25.0	11.8
	0.46	111	126	138	107	120.5	11.8
	0.92	246	251	219	295	252.8	12.5
	1.85	474	495	545	-	504.7	7.2
	3.47	742	648	665	672	681.8	6.1
	4.62	571	591	567	516	561.3	5.7
2-AA	o	31	16	27	20	23.5	28.8
	0.46	2632	2379	2622	2445	2519.5	5.0
	0.92	2330	2487	2351	2289	2364.3	3.6
	1.85	1339	1294	1264	1168	1258.8	5.6
	3.47	747	763	773	537	705.0	16.0
	4.62	526	606	568	606	576.5	6.6
2-AF	o	20	24	26	19	22.3	14.9
	0.46	691	460	-	662	604.3	20.8
	0.92	426	461	458	490	458.8	5.7
	1.85	269	245	258	242	253.5	4.9
	3.47	154	183	188	180	176.3	8.6
	4.62	141	129	133	103	126.5	13.0

TABLE 14

PROMUTAGEN ACTIVATION BY VARIOUS DOSES OF S9 PROTEIN, BATCH NO. 81.A,
STRAIN TA 98

Test substances: DMSO (40 µl/plate), WSC-I (0.1 mg/plate), B(a)P (5 µg/plate),
2-AA (2 µg/plate) and 2-AF (2 µg/plate). The test substances were applied
together with 40 µl DMSO.

date of determination: 2.Sep.81

DATE	PROTEIN CONCENTRATION		PROTEIN AMOUNT (a)	SPECIFIC AHM ACTIVITY	BACTERIAL CONTAMINATION
	UNFILTERED (g/l)	FILTERED (g/l)	(mg/plate)	(U/mg protein)	(CFU/ml)
9.Nov.81	4.0	3.1	1.5	133.7	0
10.Nov.81	4.0	3.1	1.6	130.0	0
24.Nov.81	4.0	3.1	1.6	156.3	0
25.Nov.81	4.0	2.9	1.5	119.7	0
9.Nov.81 to 25.Nov.81	-	-	-	134.9 \pm 7.7 (a)	-

TABLE 15

ANALYTICAL DATA OF S9 MIXES

S9 mixes were stored at minus 80 degrees centigrade until determination.

date of determination: 26.Nov.81

(a) MEAN \pm SE, N = 4

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE					
			1.1	1.2	2.1	2.2		
9.NOV.81	54	0.00	45.	38.	29.	30.	35.5	21.1
		0.05	100.	100.	96.	92.	97.0	3.9
		0.10	173.	182.	193.	189.	184.2	4.8
		0.15	258.	283.	260.	260.	265.2	4.5
	55	0.00	23.	38.	26.	27.	28.5	23.0
		0.05	103.	108.	96.	108.	103.7	5.5
		0.10	207.	194.	194.	214.	202.2	4.9
		0.15	271.	259.	222.	246.	249.5	8.4
24.NOV.81	56	0.00	28.	28.	30.	30.	29.0	4.0
		0.05	95.	109.	78.	113.	98.7	16.0
		0.10	171.	172.	172.	186.	175.2	4.1
		0.15	265.	258.	263.	251.	259.2	2.4
	57	0.00	24.	28.	34.	28.	28.5	14.5
		0.05	96.	122.	103.	78.	99.7	18.2
		0.10	170.	167.	182.	200.	179.7	8.3
		0.15	245.	259.	266.	259.	257.2	3.4

TABLE 16

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ T501 /2058.00

2026048964

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS		
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)	
			PLATE						
			1.1	1.2	2.1	2.2			
9.NOV.81	1	0.00	26.	29.	27.	31.	28.2	7.8	
		0.05	124.	95.	121.	120.	115.0	11.7	
		0.10	218.	206.	216.	214.	213.5	2.5	
		0.15	306.	329.	329.	313.	319.2	3.6	
	2	0.00	21.	29.	58.	30.	34.5	46.9	
		0.05	84.	110.	93.	100.	96.7	11.4	
		0.10	199.	206.	221.	217.	210.7	4.8	
		0.15	320.	332.	347.	313.	328.0	4.5	
	24.NOV.81	3	0.00	35.	44.	28.	19.	31.5	33.6
			0.05	135.	112.	119.	101.	116.7	12.2
			0.10	289.	231.	261.	245.	256.5	9.7
			0.15	401.	407.	396.	354.	389.5	6.2
4		0.00	33.	29.	37.	37.	34.0	11.3	
		0.05	127.	105.	131.	126.	122.2	9.6	
		0.10	310.	286.	288.	242.	281.5	10.1	
		0.15	393.	387.	453.	412.	411.2	7.2	

TABLE 17

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ T501 /2059.00

2026048965

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE					
			1.1	1.2	2.1	2.2		
9.NOV.81	1	0.00	23.	25.	22.	25.	23.7	6.3
		0.05	163.	153.	106.	122.	136.0	19.5
		0.10	311.	406.	333.	356.	351.5	11.6
		0.15	463.	479.	516.	499.	489.2	4.7
	2	0.00	28.	37.	32.	42.	34.7	17.5
		0.05	159.	115.	162.	178.	153.5	17.6
		0.10	363.	302.	386.	386.	359.2	11.0
		0.15	432.	625.	577.	553.	546.7	15.0
24.NOV.81	3	0.00	20.	33.	27.	26.	26.5	20.1
		0.05	188.	177.	207.	181.	188.2	7.1
		0.10	436.	404.	415.	376.	407.7	6.1
		0.15	576.	556.	673.	386.	547.7	21.8
	4	0.00	40.	24.	36.	24.	31.0	26.6
		0.05	190.	177.	168.	158.	173.2	7.8
		0.10	400.	392.	414.	407.	403.2	2.3
		0.15	602.	547.	593.	581.	580.7	4.1

TABLE 18

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR O-17-3
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ T501 /2059.00

2026048966

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE		2.1	2.2		
			1.1	1.2				
9.NOV.81	1	0.00	25.	33.	36.	38.	33.0	17.3
		0.05	81.	82.	85.	92.	85.0	5.8
		0.10	169.	182.	161.	194.	176.5	8.2
		0.15	287.	254.	279.	286.	276.5	5.6
	2	0.00	37.	34.	29.	25.	31.2	17.0
		0.05	138.	104.	104.	83.	107.2	21.2
		0.10	201.	180.	212.	200.	198.2	6.7
		0.15	335.	303.	322.	295.	313.7	5.8
24.NOV.81	3	0.00	25.	32.	26.	27.	27.5	11.3
		0.05	111.	117.	137.	109.	118.5	10.8
		0.10	256.	225.	229.	168.	219.5	16.5
		0.15	390.	364.	350.	341.	361.2	5.9
	4	0.00	38.	22.	24.	25.	27.2	26.7
		0.05	119.	75.	112.	98.	101.0	17.2
		0.10	191.	267.	211.	188.	214.2	17.1
		0.15	320.	295.	355.	317.	321.7	7.7

TABLE 19

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ T501 /2059.00

2026048967

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE					
			1.1	1.2	2.1	2.2		
9.NOV.81	1	0.00	33.	26.	21.	25.	26.2	19.0
		0.05	147.	149.	176.	143.	153.7	9.8
		0.10	245.	257.	291.	300.	273.2	9.7
		0.15	358.	433.	418.	448.	414.2	9.5
	2	0.00	33.	21.	25.	23.	25.5	20.6
		0.05	147.	137.	151.	118.	138.2	10.7
		0.10	337.	342.	385.	334.	349.5	6.8
		0.15	463.	530.	513.	428.	483.5	9.7
24.NOV.81	3	0.00	37.	28.	32.	26.	30.7	15.8
		0.05	152.	158.	145.	162.	154.2	4.8
		0.10	380.	315.	314.	210.	329.7	10.2
		0.15	419.	487.	494.	434.	458.5	8.2
	4	0.00	43.	24.	30.	30.	31.7	25.2
		0.05	177.	156.	180.	171.	171.0	6.2
		0.10	339.	305.	353.	376.	343.2	8.7
		0.15	490.	460.	516.	508.	493.5	5.0

TABLE 2o

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ T501. /2059.00

2026048968

A-72059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE					
			1.1	1.2	2.1	2.2		
9.NOV.81	1	0.00	35.	23.	28.	18.	26.0	27.9
		0.05	97.	89.	113.	111.	102.5	11.2
		0.10	241.	241.	258.	213.	230.7	9.9
		0.15	308.	280.	315.	349.	313.0	9.1
	2	0.00	30.	42.	29.	20.	30.2	29.9
		0.05	100.	105.	94.	101.	100.0	4.5
		0.10	236.	226.	208.	228.	224.5	5.3
		0.15	291.	326.	322.	389.	332.0	12.4
24.NOV.81	3	0.00	27.	28.	28.	32.	28.7	7.7
		0.05	114.	126.	109.	113.	115.5	6.3
		0.10	215.	215.	298.	219.	236.7	17.3
		0.15	389.	362.	328.	358.	359.2	7.0
	4	0.00	35.	34.	37.	27.	33.5	13.5
		0.05	117.	135.	110.	107.	117.2	10.7
		0.10	179.	186.	255.	242.	215.5	17.9
		0.15	280.	255.	370.	316.	305.2	16.3

TABLE 21

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ T501 /2059.00

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.1		DIL.SUSP.2		MEAN	RSD (%)
			PLATE					
			1.1	1.2	2.1	2.2		
9.NOV.81	1	0.00	27.	28.	21.	28.	26.0	12.9
		0.05	151.	72.	118.	89.	107.5	32.2
		0.10	322.	289.	306.	280.	299.2	6.2
		0.15	501.	506.	437.	485.	482.2	6.5
	2	0.00	34.	34.	30.	32.	32.5	5.9
		0.05	-	-	137.	136.	136.5	0.5
		0.10	299.	301.	340.	330.	317.5	6.5
		0.15	426.	439.	490.	486.	460.2	7.1
24.NOV.81	3	0.00	28.	31.	24.	38.	30.2	19.5
		0.05	178.	119.	159.	129.	146.2	18.6
		0.10	356.	284.	325.	303.	317.0	9.8
		0.15	526.	479.	541.	457.	500.7	7.9
	4	0.00	25.	29.	25.	30.	27.2	9.7
		0.05	155.	163.	132.	106.	139.0	18.4
		0.10	329.	226.	256.	278.	272.2	15.9
		0.15	457.	399.	477.	439.	443.0	7.5

TABLE 22

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ T501 /2059.00

2026048970

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4		MEAN	RSD (%)
			PLATE 3.1	3.2	4.1	4.2		
10.NOV.81	54	0.00	108.	118.	105.	123.	113.5	7.4
		0.05	153.	153.	166.	139.	152.7	7.2
		0.10	176.	219.	192.	192.	194.7	9.2
		0.15	224.	218.	236.	221.	224.7	3.5
	55	0.00	88.	113.	113.	117.	107.7	12.3
		0.05	141.	146.	152.	143.	145.5	3.3
		0.10	209.	195.	186.	183.	193.2	6.0
		0.15	229.	242.	231.	192.	223.5	9.7
25.NOV.81	56	0.00	111.	104.	116.	129.	115.0	9.2
		0.05	140.	137.	149.	155.	145.2	5.7
		0.10	167.	165.	169.	168.	167.2	1.0
		0.15	212.	216.	210.	213.	212.7	1.2
	57	0.00	110.	108.	117.	118.	113.2	4.4
		0.05	154.	148.	168.	146.	154.0	6.5
		0.10	175.	174.	207.	177.	183.2	8.7
		0.15	195.	210.	202.	216.	205.7	4.5

TABLE 23

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION; STRAIN TA 100

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4		MEAN	RSD (%)
			PLATE 3.1	3.2	4.1	4.2		
10.NOV.81	1	0.00	108.	142.	117.	110.	119.2	13.1
		0.05	162.	163.	161.	149.	158.7	4.1
		0.10	223.	199.	181.	174.	194.2	11.3
		0.15	217.	263.	215.	227.	230.5	9.7
	2	0.00	105.	120.	127.	104.	114.0	10.0
		0.05	149.	158.	205.	151.	165.7	16.0
		0.10	209.	227.	214.	183.	208.2	8.9
		0.15	261.	243.	224.	220.	237.0	8.0
25.NOV.81	3	0.00	110.	112.	125.	116.	115.7	5.7
		0.05	178.	163.	181.	152.	168.5	8.0
		0.10	199.	206.	218.	228.	212.7	6.0
		0.15	262.	243.	238.	254.	249.2	4.3
	4	0.00	122.	96.	108.	125.	112.7	11.9
		0.05	163.	165.	172.	166.	166.5	2.3
		0.10	192.	203.	241.	233.	217.2	10.8
		0.15	282.	256.	265.	237.	260.0	7.2

TABLE 24

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION; STRAIN TA 100

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4		MEAN	RSD (%)
			PLATE					
			3.1	3.2	4.1	4.2		
10.NOV.81	1	0.00	108.	123.	119.	89.	109.7	13.9
		0.05	171.	154.	191.	173.	172.2	8.8
		0.10	243.	240.	252.	261.	249.0	3.8
		0.15	300.	302.	320.	315.	309.2	3.2
	2	0.00	109.	116.	113.	115.	113.2	2.7
		0.05	178.	167.	215.	169.	182.2	12.3
		0.10	307.	248.	223.	230.	252.0	15.1
		0.15	362.	324.	324.	336.	336.5	5.3
25.NOV.81	3	0.00	110.	129.	135.	125.	124.7	8.5
		0.05	169.	185.	217.	166.	184.2	12.7
		0.10	279.	266.	265.	226.	259.0	8.8
		0.15	340.	323.	321.	336.	330.0	2.9
	4	0.00	103.	116.	117.	132.	117.0	10.1
		0.05	195.	190.	205.	194.	196.0	3.3
		0.10	175.	193.	294.	227.	222.2	23.6
		0.15	283.	308.	331.	316.	309.5	6.5

TABLE 25

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR D-17-3
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ T501 /2059.00

2026048973

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4			
			PLATE 3.1	3.2	4.1	4.2	MEAN	RSD (%)
10.NOV.81	1	0.00	123.	103.	131.	126.	120.7	10.2
		0.05	177.	161.	172.	162.	168.0	4.6
		0.10	184.	196.	231.	252.	215.7	14.5
		0.15	250.	250.	274.	237.	252.7	6.1
	2	0.00	101.	103.	119.	116.	109.7	8.3
		0.05	155.	150.	176.	256.	184.2	26.7
		0.10	203.	199.	174.	216.	198.0	8.9
		0.15	235.	211.	241.	253.	235.0	7.5
25.NOV.81	3	0.00	123.	-	105.	126.	118.0	9.6
		0.05	185.	191.	155.	161.	173.0	10.2
		0.10	221.	244.	191.	219.	218.7	9.9
		0.15	269.	269.	263.	277.	269.5	2.1
	4	0.00	120.	116.	114.	94.	111.0	10.5
		0.05	166.	187.	158.	164.	168.7	7.5
		0.10	200.	221.	174.	192.	196.7	9.9
		0.15	259.	243.	260.	261.	255.7	3.3

TABLE 26

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION; STRAIN TA 100

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4		MEAN	RSD (%)
			PLATE 3.1	3.2	4.1	4.2		
10.NOV.81	1	0.00	126.	102.	106.	118.	113.0	9.7
		0.05	185.	146.	145.	135.	152.7	14.4
		0.10	271.	230.	220.	192.	228.2	14.3
		0.15	287.	281.	277.	272.	279.2	2.3
	2	0.00	115.	118.	103.	105.	110.2	6.7
		0.05	150.	163.	151.	159.	155.7	4.0
		0.10	241.	277.	226.	209.	238.2	12.2
		0.15	309.	259.	284.	278.	282.5	7.3
25.NOV.81	3	0.00	123.	106.	124.	102.	113.7	10.0
		0.05	179.	166.	177.	182.	176.0	4.0
		0.10	248.	227.	238.	190.	225.7	11.2
		0.15	290.	293.	326.	276.	296.2	7.1
	4	0.00	120.	120.	112.	93.	111.2	11.4
		0.05	189.	175.	181.	189.	183.5	3.7
		0.10	249.	238.	260.	187.	233.5	13.8
		0.15	303.	305.	292.	278.	294.5	6.2

TABLE 27

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4			
			PLATE		4.1	4.2	MEAN	RSD (%)
			3.1	3.2				
10.NOV.81	1	0.00	117.	99.	129.	98.	110.7	13.5
		0.05	152.	147.	157.	134.	147.5	6.7
		0.10	209.	226.	230.	208.	218.2	5.2
		0.15	253.	243.	262.	249.	251.7	3.2
	2	0.00	103.	101.	122.	115.	110.2	9.1
		0.05	149.	123.	127.	129.	132.0	8.8
		0.10	208.	185.	205.	188.	196.5	5.9
		0.15	251.	219.	254.	215.	234.7	8.8
25.NOV.81	3	0.00	105.	121.	90.	112.	107.0	12.2
		0.05	157.	159.	182.	155.	163.2	7.7
		0.10	215.	193.	189.	194.	197.7	5.9
		0.15	226.	220.	234.	219.	224.7	3.1
	4	0.00	95.	110.	109.	101.	103.7	6.8
		0.05	165.	163.	175.	138.	160.2	9.8
		0.10	201.	188.	196.	175.	190.0	6.0
		0.15	250.	233.	220.	215.	229.5	6.8

TABLE 28

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION; STRAIN TA 100

A-/2059.00

DATE	CON- DEN- SATE BATCH	DOSE (MG/ PLATE)	REVERTANTS PER PLATE				STATISTICAL PARAMETERS	
			DIL.SUSP.3		DIL.SUSP.4		MEAN	RSD (%)
			PLATE 3.1	3.2	4.1	4.2		
10.NOV.81	1	0.00	95.	101.	103.	106.	101.2	4.6
		0.05	175.	142.	174.	143.	158.5	11.7
		0.10	234.	216.	202.	174.	206.5	12.3
		0.15	286.	257.	259.	229.	257.7	9.0
	2	0.00	105.	123.	111.	128.	116.7	9.1
		0.05	151.	158.	159.	147.	153.7	3.7
		0.10	206.	223.	205.	189.	205.7	6.8
		0.15	279.	252.	280.	279.	272.5	5.0
25.NOV.81	3	0.00	107.	115.	103.	115.	110.0	5.5
		0.05	143.	164.	136.	152.	148.7	8.1
		0.10	207.	193.	191.	213.	201.0	5.3
		0.15	224.	268.	209.	259.	240.0	11.7
	4	0.00	98.	111.	96.	100.	101.2	6.6
		0.05	131.	149.	143.	153.	144.0	6.7
		0.10	211.	205.	179.	232.	206.7	10.6
		0.15	237.	267.	264.	247.	253.7	5.6

TABLE 29

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100

CIGARETTE	DATE OF PLATE INCORPORATION MUTAGENICITY ASSAY	NUMBER OF COLONIES/ PLATE	NUMBER OF HISTIDINE PROTOTROPHS/ 10 COLONIES ASSAYED
2R1	9.Nov.81	283	10
	24.Nov.81	258	10
LEAR 0-17-2	9.Nov.81	329	10
	24.Nov.81	407	10
LEAR 0-17-3	9.Nov.81	479	10
	24.Nov.81	556	10
LEAR 2-17-2	9.Nov.81	254	10
	24.Nov.81	364	10
LEAR 2-17-3	9.Nov.81	433	10
	24.Nov.81	487	10
LEAR 4-17-2	9.Nov.81	280	10
	24.Nov.81	362	10
LEAR 4-17-3	9.Nov.81	506	10
	24.Nov.81	479	10

TABLE 30

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF TA 98

colonies from plate incorporation mutagenicity assay plates

dates of determinations: 11.Nov.81
26.Nov.81

2026048978

CIGARETTE	DATE OF PLATE INCORPORATION MUTAGENICITY ASSAY	NUMBER OF COLONIES/ PLATE	NUMBER OF HISTIDINE PROTOTROPHS/ 100 COLONIES ASSAYED
2R1	10.Nov.81	218	10
	25.Nov.81	216	10
LEAR 0-17-2	10.Nov.81	263	10
	25.Nov.81	243	10
LEAR 0-17-3	10.Nov.81	302	10
	25.Nov.81	323	10
LEAR 2-17-2	10.Nov.81	250	10
	25.Nov.81	269	10
LEAR 2-17-3	10.Nov.81	281	10
	25.Nov.81	293	10
LEAR 4-17-2	10.Nov.81	243	10
	25.Nov.81	220	10
LEAR 4-17-3	10.Nov.81	257	10
	25.Nov.81	268	10

TABLE 31

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF TA 100
colonies from plate incorporation mutagenicity assay plates

dates of determinations: 12.Nov.81
27.Nov.81

2026048979

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	32.0	23.5		
	0.05	8	100.3	5.7		
	0.10	8	193.2	6.7		
	0.15	8	257.3	7.0	1538.	0.989
24.NOV.81	0.00	8	28.7	9.8		
	0.05	8	99.2	15.9		
	0.10	8	177.5	6.3		
	0.15	8	258.2	2.8	1533.	0.993
9.NOV.81	0.00	16	30.4	18.9		
24.NOV.81	0.05	16	99.8	11.5		
	0.10	16	185.4	7.7		
	0.15	16	257.8	5.1	1536.	0.991

TABLE 32

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ F501 /2058.00

2026048980

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	31.3	35.7		
	0.05	8	105.8	14.1		
	0.10	8	212.1	3.6		
	0.15	8	323.6	4.1	1966.	0.991
24.NOV.81	0.00	8	32.7	22.9		
	0.05	8	119.5	10.4		
	0.10	8	269.0	10.5		
	0.15	8	400.3	6.9	2505.	0.985
9.NOV.81	0.00	16	32.1	28.8		
24.NOV.81	0.05	16	112.7	13.3		
	0.10	16	240.6	14.8		
	0.15	16	362.0	12.4	2235.	0.970

TABLE 33

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ F501 /2059.00

2026048981

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	29.2	24.5		
	0.05	8	144.7	18.3		
	0.10	8	355.3	10.5		
	0.15	8	518.0	12.3	3354.	0.976
24.NOV.81	0.00	8	28.7	23.9		
	0.05	8	180.7	8.2		
	0.10	8	405.5	4.4		
	0.15	8	564.2	14.5	3662.	0.979
9.NOV.81	0.00	16	29.0	23.4		
24.NOV.81	0.05	16	162.8	17.1		
	0.10	16	380.4	10.1		
	0.15	16	541.1	13.8	3508.	0.973

TABLE 34

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048982

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	32.1	16.2		
	0.05	8	96.1	20.1		
	0.10	8	187.3	9.3		
	0.15	8	295.1	8.6	1760.	0.979
24.NOV.81	0.00	8	27.3	18.9		
	0.05	8	109.7	16.3		
	0.10	8	216.8	15.8		
	0.15	8	341.5	8.8	2099.	0.977
9.NOV.81	0.00	16	29.8	18.8		
24.NOV.81	0.05	16	102.9	18.8		
	0.10	16	202.1	15.0		
	0.15	16	318.3	11.3	1930.	0.970

TABLE 35

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ F501 /2059.00

2026048983

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	25.8	18.4		
	0.05	8	146.0	11.0		
	0.10	8	311.3	15.1		
	0.15	8	448.8	12.1	2869.	0.976
24.NOV.81	0.00	8	31.2	19.7		
	0.05	8	162.6	7.6		
	0.10	8	336.5	9.0		
	0.15	8	476.0	7.3	3016.	0.990
9.NOV.81 24.NOV.81	0.00	16	28.6	21.0		
	0.05	16	154.3	10.6		
	0.10	16	323.9	12.4		
	0.15	16	462.4	10.0	2942.	0.981

TABLE 36

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ F501 /2059.00

2026048984

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	28.1	28.2		
	0.05	8	101.2	8.1		
	0.10	8	227.6	7.5		
	0.15	8	322.5	10.6	2019.	0.982
24.NOV.81	0.00	8	31.1	13.4		
	0.05	8	116.3	8.2		
	0.10	8	226.1	17.0		
	0.15	8	332.2	14.0	2026.	0.968
9.NOV.81 24.NOV.81	0.00	16	29.6	21.3		
	0.05	16	108.8	10.7		
	0.10	16	226.9	12.7		
	0.15	16	327.4	12.2	2023.	0.975

TABLE 37

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION; STRAIN TA 98

502.00/ F501 /2059.00

2026048985

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
9.NOV.81	0.00	8	29.2	14.7		
	0.05	6	117.1	26.2		
	0.10	8	308.3	6.7		
	0.15	8	471.2	6.8	3014.	0.984
24.NOV.81	0.00	8	28.7	15.7		
	0.05	8	142.6	17.3		
	0.10	8	294.6	14.4		
	0.15	8	471.8	9.7	2963.	0.978
9.NOV.81	0.00	16	29.0	14.7		
24.NOV.81	0.05	14	131.7	22.3		
	0.10	16	301.5	10.9		
	0.15	16	471.6	8.1	2988.	0.981

TABLE 38

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048986

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	110.6	9.7		
	0.05	8	149.1	5.9		
	0.10	8	194.0	7.2		
	0.15	8	224.1	6.8	771.	0.963
25.NOV.81	0.00	8	114.1	6.7		
	0.05	8	149.6	6.5		
	0.10	8	175.2	7.7		
	0.15	8	209.2	3.5	622.	0.965
10.NOV.81	0.00	16	112.4	8.2		
25.NOV.81	0.05	16	149.4	6.0		
	0.10	16	184.6	8.9		
	0.15	16	216.7	6.4	696.	0.954

TABLE 39

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2058.00

2026048987

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	116.6	11.1		
	0.05	8	162.2	11.2		
	0.10	8	201.2	10.0		
	0.15	8	233.7	8.3	781.	0.931
25.NOV.81	0.00	8	114.2	8.7		
	0.05	8	167.5	5.5		
	0.10	8	215.0	8.2		
	0.15	8	254.6	6.0	937.	0.970
10.NOV.81	0.00	16	115.4	9.7		
25.NOV.81	0.05	16	164.9	8.6		
	0.10	16	208.1	9.4		
	0.15	16	244.2	8.2	859.	0.946

TABLE 40

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048988

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	111.5	9.3	1415.	0.974
	0.05	8	177.2	10.4		
	0.10	8	250.5	10.3		
	0.15	8	322.8	6.1		
25.NOV.81	0.00	8	120.8	9.3	1294.	0.948
	0.05	8	190.1	9.0		
	0.10	8	240.6	17.6		
	0.15	8	319.7	5.7		
10.NOV.81	0.00	16	116.2	9.9	1354.	0.961
25.NOV.81	0.05	16	183.7	10.0		
	0.10	16	245.6	13.9		
	0.15	16	321.3	5.7		

TABLE 41

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048989

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	115.2	10.1		
	0.05	8	176.1	19.1		
	0.10	8	206.8	12.3		
	0.15	8	243.8	7.4	833.	0.894
25.NOV.81	0.00	7	114.0	9.8		
	0.05	8	170.8	8.4		
	0.10	8	207.7	10.8		
	0.15	8	262.6	3.8	964.	0.962
10.NOV.81	0.00	15	114.7	9.6		
25.NOV.81	0.05	16	173.5	14.5		
	0.10	16	207.3	11.2		
	0.15	16	253.3	6.7	897.	0.928

TABLE 42

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048990

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	111.6	7.9	1173.	0.961
	0.05	8	154.2	9.8		
	0.10	8	233.2	12.5		
	0.15	8	280.8	5.1		
25.NOV.81	0.00	8	112.5	10.0	1197.	0.971
	0.05	8	179.7	4.2		
	0.10	8	229.6	11.8		
	0.15	8	295.3	5.4		
10.NOV.81	0.00	16	112.1	8.7	1185.	0.964
25.NOV.81	0.05	16	167.0	10.5		
	0.10	16	231.4	11.8		
	0.15	16	288.1	5.7		

TABLE 43

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048991

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	110.5	10.7		
	0.05	8	139.7	9.3		
	0.10	8	207.3	7.6		
	0.15	8	243.2	7.0	932.	0.957
25.NOV.81	0.00	8	105.3	9.4		
	0.05	8	161.7	8.2		
	0.10	8	193.8	5.9		
	0.15	8	227.1	5.0	795.	0.962
10.NOV.81	0.00	16	107.9	10.0		
25.NOV.81	0.05	16	150.8	11.3		
	0.10	16	200.6	7.5		
	0.15	16	235.2	6.9	863.	0.956

TABLE 44

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048992

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	109.0	10.3		
	0.05	8	156.1	8.3		
	0.10	8	206.1	9.2		
	0.15	8	265.1	7.3	1037.	0.967
25.NOV.81	0.00	8	105.6	7.1		
	0.05	8	146.3	7.1		
	0.10	8	203.8	7.9		
	0.15	8	246.8	8.9	962.	0.965
10.NOV.81	0.00	16	107.3	8.8		
25.NOV.81	0.05	16	151.3	8.2		
	0.10	16	205.0	8.3		
	0.15	16	256.0	8.6	1000.	0.963

TABLE 45

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048993

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	116.6	11.1		
	0.05	8	162.2	11.2		
	0.10	8	201.2	10.0		
	0.15	8	233.7	8.3	781.	0.931
25.NOV.81	0.00	8	114.2	8.7		
	0.05	8	167.5	5.5		
	0.10	8	215.0	8.2		
	0.15	8	254.6	6.0	937.	0.970
10.NOV.81	0.00	16	115.4	9.7		
25.NOV.81	0.05	16	164.9	8.6		
	0.10	16	208.1	9.4		
	0.15	16	244.2	8.2	859.	0.946

TABLE 4o

SPECIFIC MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048994

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	111.5	9.3	1415.	0.974
	0.05	8	177.2	10.4		
	0.10	8	250.5	10.3		
	0.15	8	322.8	6.1		
25.NOV.81	0.00	8	120.8	9.3	1294.	0.948
	0.05	8	190.1	9.0		
	0.10	8	240.6	17.6		
	0.15	8	319.7	5.7		
10.NOV.81	0.00	16	116.2	9.9	1354.	0.961
25.NOV.81	0.05	16	183.7	10.0		
	0.10	16	245.6	13.9		
	0.15	16	321.3	5.7		

TABLE 41

SPECIFIC MUTAGENICITY OF MSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048995

A- /2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	115.2	10.1	833.	0.894
	0.05	8	176.1	19.1		
	0.10	8	206.8	12.3		
	0.15	8	243.8	7.4		
25.NOV.81	0.00	7	114.0	9.8	964.	0.962
	0.05	8	170.8	8.4		
	0.10	8	207.7	10.8		
	0.15	8	262.6	3.8		
10.NOV.81	0.00	15	114.7	9.6	897.	0.928
25.NOV.81	0.05	16	173.5	14.5		
	0.10	16	207.3	11.2		
	0.15	16	253.3	6.7		

TABLE 42

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048996

A-/2059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	111.6	7.9	1173.	0.961
	0.05	8	154.2	9.8		
	0.10	8	233.2	12.5		
	0.15	8	280.8	5.1		
25.NOV.81	0.00	8	112.5	10.0	1197.	0.971
	0.05	8	179.7	4.2		
	0.10	8	229.6	11.8		
	0.15	8	295.3	5.4		
10.NOV.81	0.00	16	112.1	8.7	1185.	0.964
25.NOV.81	0.05	16	167.0	10.5		
	0.10	16	231.4	11.8		
	0.15	16	288.1	5.7		

TABLE 43

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048997

A-72059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	110.5	10.7	932.	0.957
	0.05	8	139.7	9.3		
	0.10	8	207.3	7.6		
	0.15	8	243.2	7.0		
25.NOV.81	0.00	8	105.3	9.4	795.	0.962
	0.05	8	161.7	8.2		
	0.10	8	193.8	5.9		
	0.15	8	227.1	5.0		
10.NOV.81	0.00	16	107.9	10.0	863.	0.956
25.NOV.81	0.05	16	150.8	11.3		
	0.10	16	200.6	7.5		
	0.15	16	235.2	6.9		

TABLE 44

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION; STRAIN TA 100

502.10% F501 /2059.00

2026048998

A-72059.00

DATE	DOSE (MG/ PLATE)	REVERTANTS PER PLATE STATISTICAL PARAMETERS			REGR. COEFF. (1/MG)	CORREL. COEFF.
		N	MEAN	RSD (%)		
10.NOV.81	0.00	8	109.0	10.3		
	0.05	8	156.1	8.3		
	0.10	8	206.1	9.2		
	0.15	8	265.1	7.3	1037.	0.967
25.NOV.81	0.00	8	105.6	7.1		
	0.05	8	146.3	7.1		
	0.10	8	203.8	7.9		
	0.15	8	246.8	8.9	962.	0.965
10.NOV.81	0.00	16	107.3	8.8		
25.NOV.81	0.05	16	151.3	8.2		
	0.10	16	205.0	8.3		
	0.15	16	256.0	8.6	1000.	0.963

TABLE 45

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION; STRAIN TA 100

502.10/ F501 /2059.00

2026048999

A	B	C	D	E	
CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)				
	ASSAY				
	1	2	MEAN	RELATIVE DIFFERENCE	
			$((B + C)/2)$	$((B - C)/D)$.GE.0.25 ABSOLUTE
2R1	1538	1533	1536	0.00	0
LEAR 0-17-2	1966	2505	2236	0.24	0
LEAR 0-17-3	3354	3662	3508	0.09	0
LEAR 2-17-2	1760	2099	1930	0.18	0
LEAR 2-17-3	2869	3016	2943	0.05	0
LEAR 4-17-2	2019	2026	2023	0.00	0
LEAR 4-17-3	3014	2963	2989	0.02	0

TABLE 46

STATISTICAL SIGNIFICANCE BETWEEN 2 INDEPENDENT ASSAYS,
STRAIN TA 98

2026043000

A	B	C	D	E	
CIGARETTE:	SPECIFIC MUTAGENICITY (rev./mg)				
	ASSAY				
	1	2	MEAN ((B + C)/2)	RELATIVE DIFFERENCE ((B - C)/D) .GE.0.25 ABSOLUTE	
2R1	771	622	697	0.21	o
LEAR 0-17-2	781	937	859	0.18	o
LEAR 0-17-3	1415	1294	1355	0.09	o
LEAR 2-17-2	833	964	899	0.15	o
LEAR 2-17-3	1173	1197	1185	0.02	o
LEAR 4-17-2	932	795	864	0.16	o
LEAR 4-17-3	1037	962	1000	0.08	o

TABLE 47

STATISTICAL SIGNIFICANCE BETWEEN 2 INDEPENDENT ASSAYS,
STRAIN TA 100

2026049001

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)	CORREL. COEFF.	RELATIVE SPEC. MUTAGENICITY (o/o)
2R1	1536	0.991	100.0 (a)
LEAR 0-17-2	2235	0.970	145.5
LEAR 0-17-3	3508	0.973	228.4
LEAR 2-17-2	1930	0.970	125.7
LEAR 2-17-3	2942	0.981	191.5
LEAR 4-17-2	2023	0.975	131.7
LEAR 4-17-3	2988	0.981	194.5

TABLE 48

SPECIFIC AND RELATIVE SPECIFIC MUTAGENICITY OF WSC-I,
STRAIN TA 98

(a) specific mutagenicity of WSC-I of 2R1 set to 100 o/o

2026043002

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)	CORREL. COEFF.	RELATIVE SPEC. MUTAGENICITY (o/o)
2R1	696	0.954	100.0 (a)
LEAR 0-17-2	859	0.946	123.4
LEAR 0-17-3	1354	0.961	194.5
LEAR 2-17-2	897	0.928	128.9
LEAR 2-17-3	1185	0.964	170.3
LEAR 4-17-2	863	0.956	124.0
LEAR 4-17-3	1000	0.963	143.7

TABLE 49

SPECIFIC AND RELATIVE SPECIFIC MUTAGENICITY OF WSC-I,
STRAIN TA 100

(a) specific mutagenicity of WSC-I of 2R1 set to 100 o/o

2026043003

CIGARETTE TYPE	2R1	LEAR o-17-2	LEAR o-17-3	LEAR 2-17-2	LEAR 2-17-3	LEAR 4-17-2	LEAR 4-17-3
2R1	-	1	1	1	1	1	1
LEAR o-17-2		-	1	o	1	o	1
LEAR o-17-3			-	2	2	2	2
LEAR 2-17-2				-	1	o	1
LEAR 2-17-3					-	2	o
LEAR 4-17-2						-	1
LEAR 4-17-3							-

TABLE 5o

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE
SPECIFIC MUTAGENICITIES OF 2 INDIVIDUAL CIGARETTES,
STRAIN TA 98

- o : no statistically significant difference
- 1 : mutagenicity of cigarette given in headline
higher than mutagenicity of cigarette given
in column
- 2 : mutagenicity of cigarette given in headline
lower than mutagenicity of cigarette given
in column

2026043004

CIGARETTE TYPE	2R1	LEAR 0-17-2	LEAR 0-17-3	LEAR 2-17-2	LEAR 2-17-3	LEAR 4-17-2	LEAR 4-17-3
2R1	-	1	1	1	1	1	1
LEAR 0-17-2		-	1	0	1	0	0
LEAR 0-17-3			-	2	0	2	2
LEAR 2-17-2				-	1	0	0
LEAR 2-17-3					-	2	2
LEAR 4-17-2						-	0
LEAR 4-17-3							-

TABLE 51

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE
SPECIFIC MUTAGENICITIES OF 2 INDIVIDUAL CIGARETTES,
STRAIN TA 100

- 0 : no statistically significant difference
- 1 : mutagenicity of cigarette given in headline
higher than mutagenicity of cigarette given
in column
- 2 : mutagenicity of cigarette given in headline
lower than mutagenicity of cigarette given
in column

2026043005

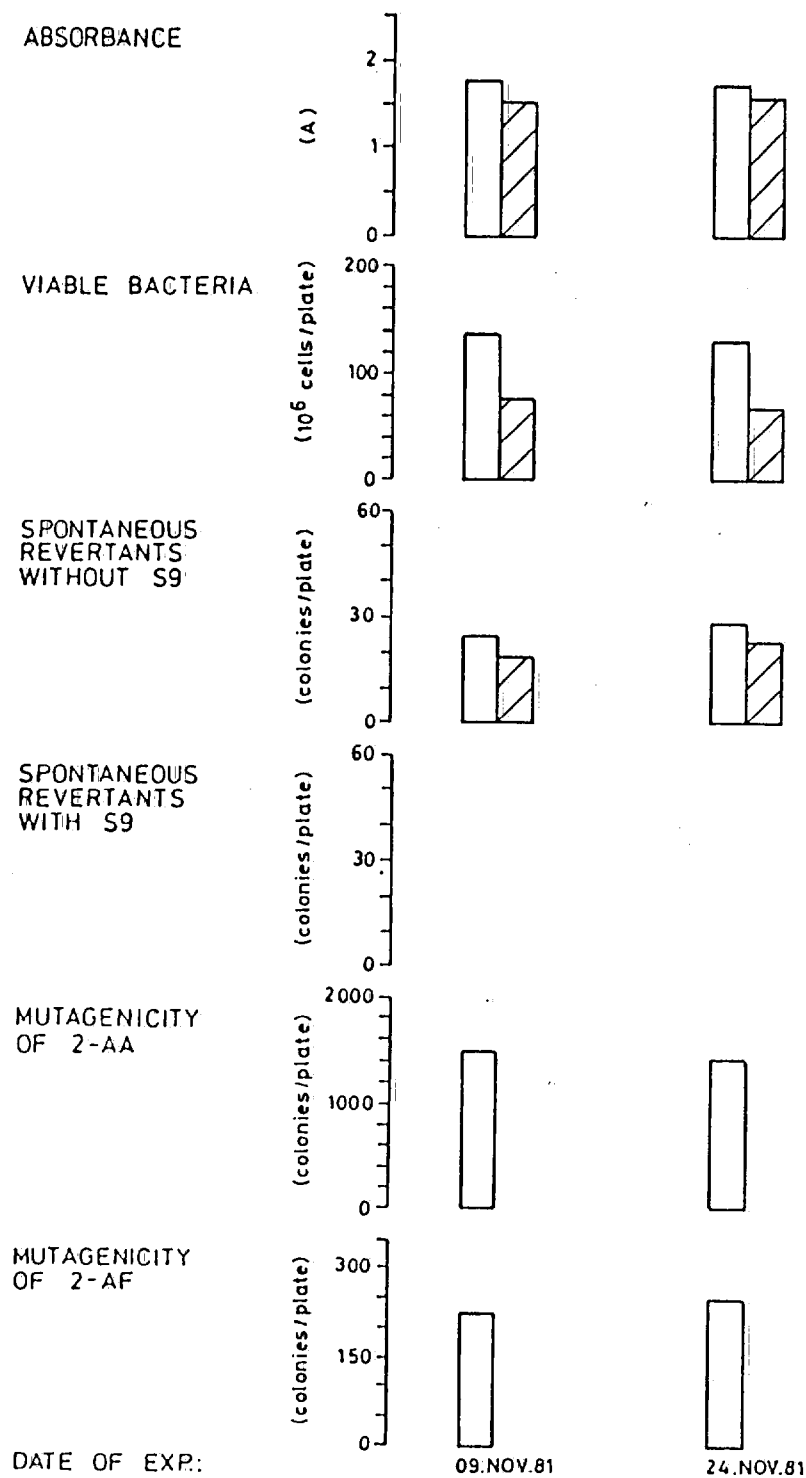


FIGURE 1

PROPERTIES OF TA 98 SUSPENSION CULTURES, test performed at the beginning (□) and at the end (▨) of each mutagenicity assay (see TABLES 5, 6 and 9).

2026049006

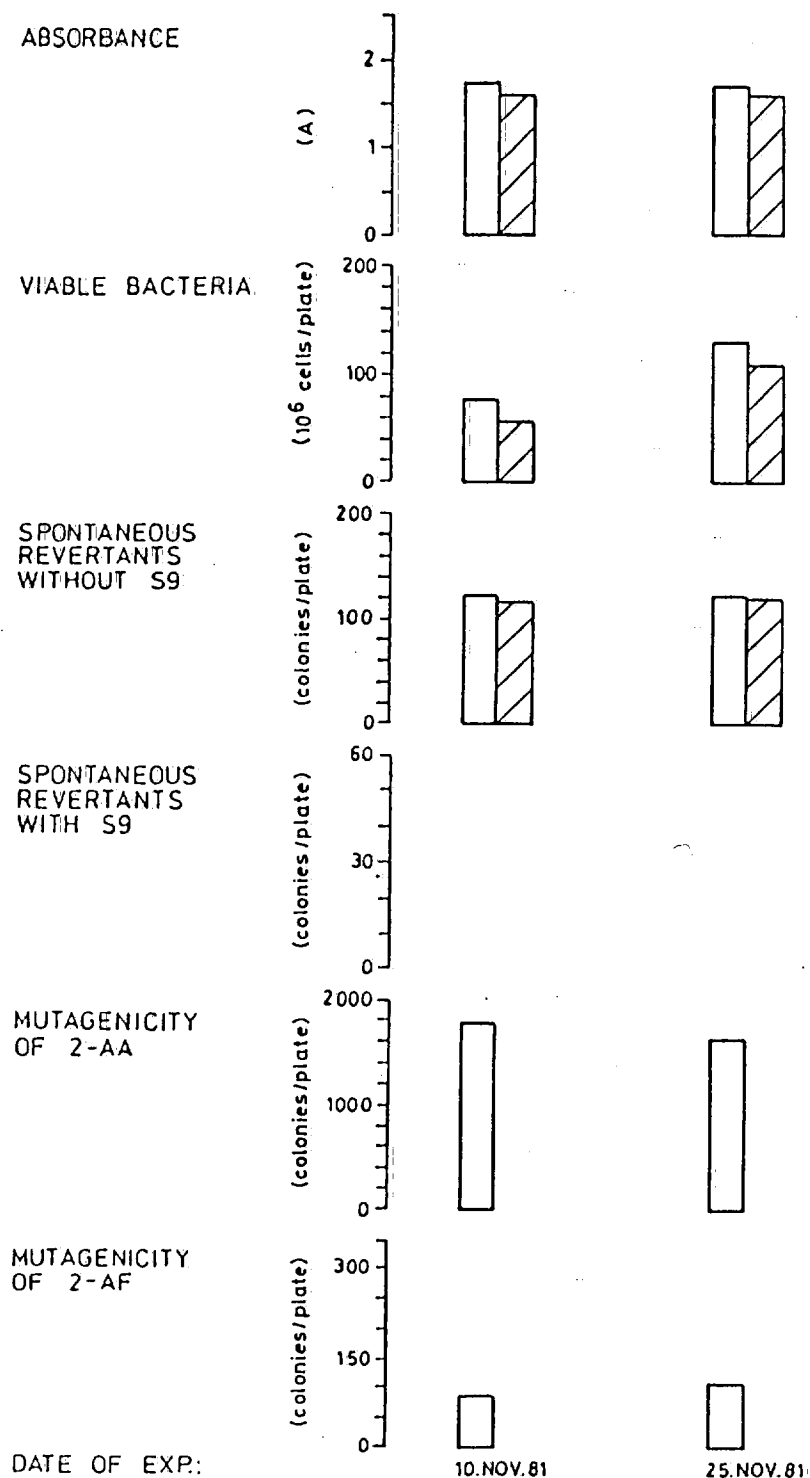


FIGURE 2

PROPERTIES OF TA 100 SUSPENSION CULTURES, test performed at the beginning (\square) and at the end (▨) of each mutagenicity assay (see TABLES 7, 8 and 10)

2026049007

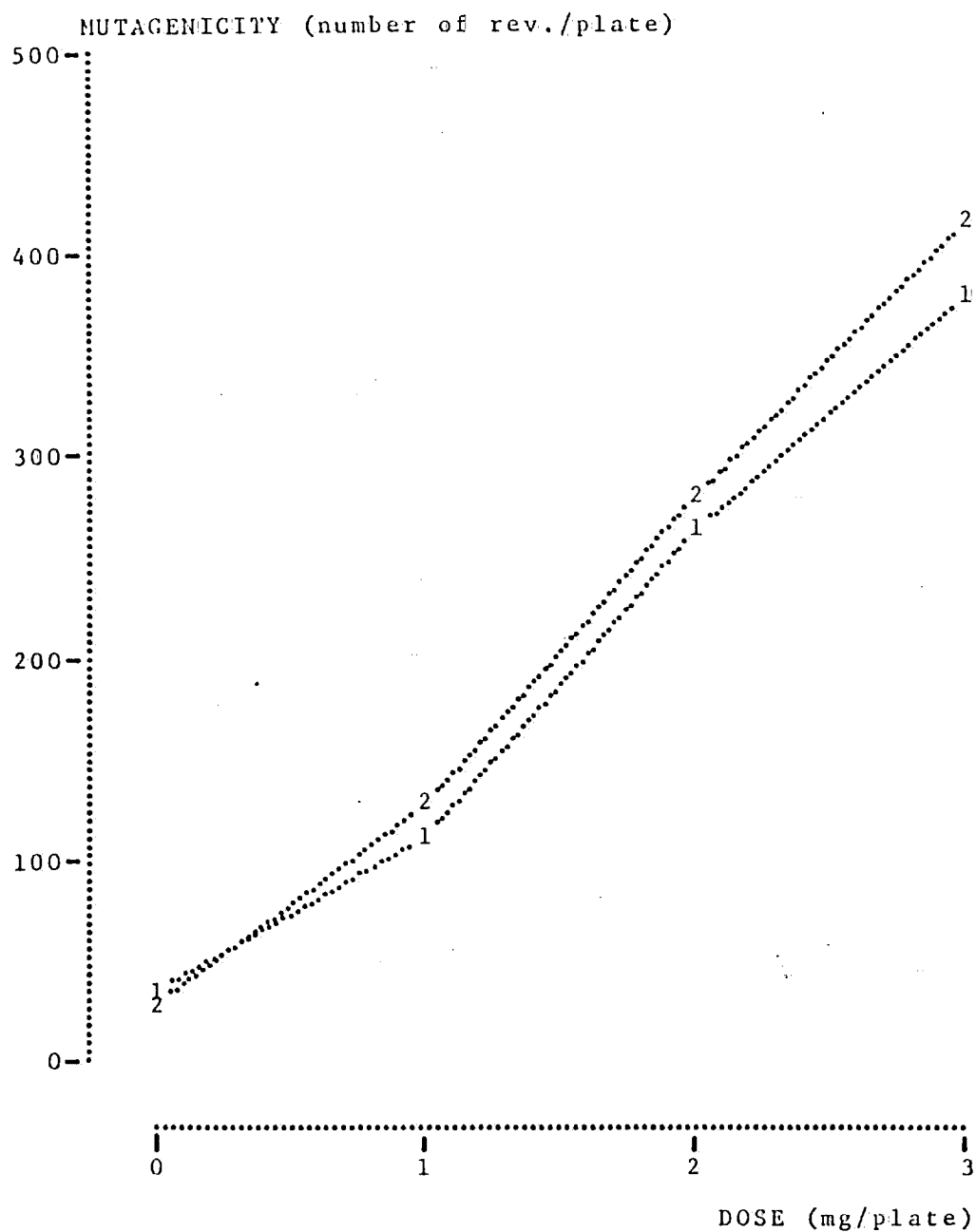


FIGURE 3

MUTAGENICITY OF 2-AMINOFLUORENE WITH S9 ACTIVATION, STRAIN TA 98
(see TABLE 11)

2026043008

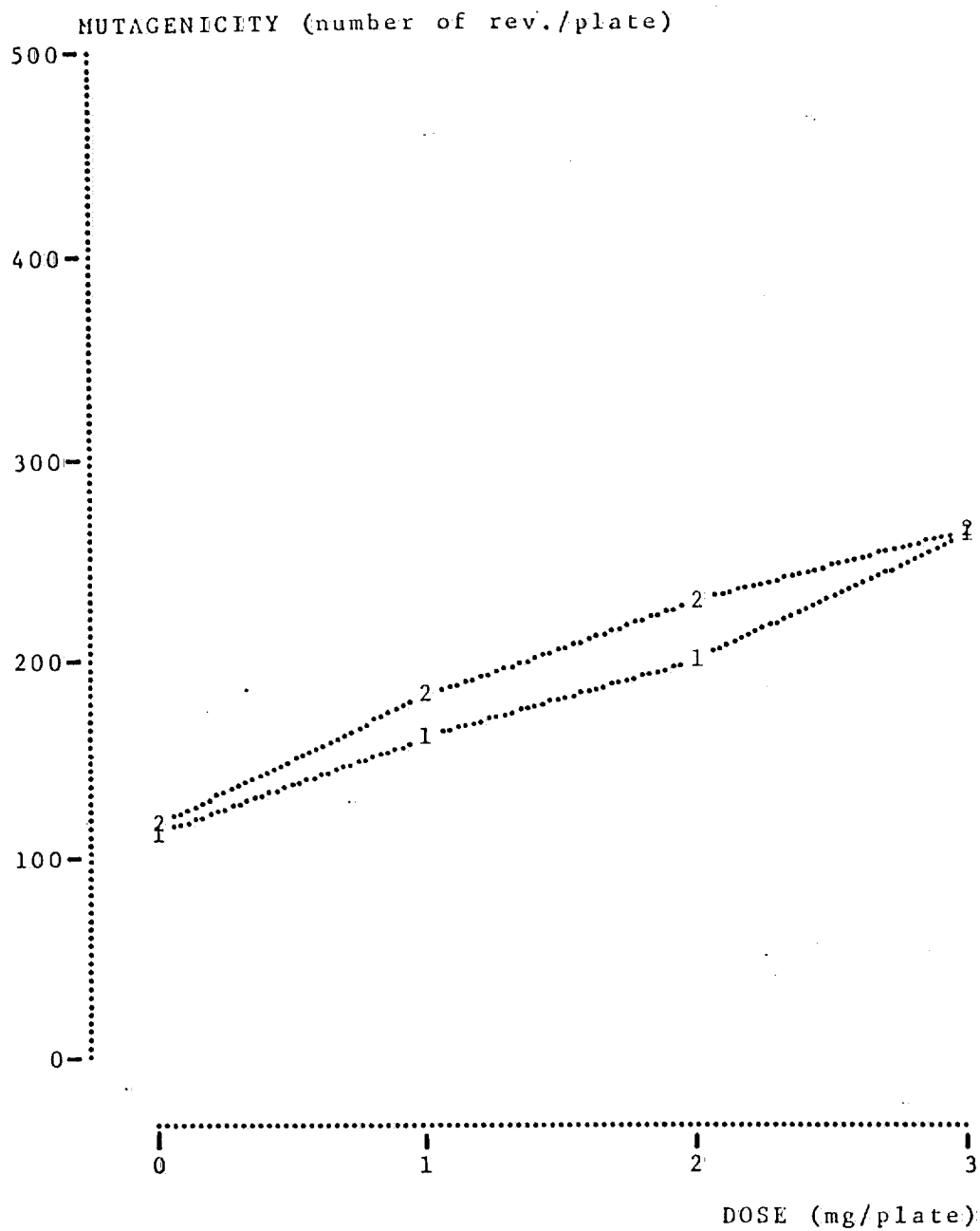


FIGURE 4

MUTAGENICITY OF 2-AMINOFLUORENE WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 12)

2026043009

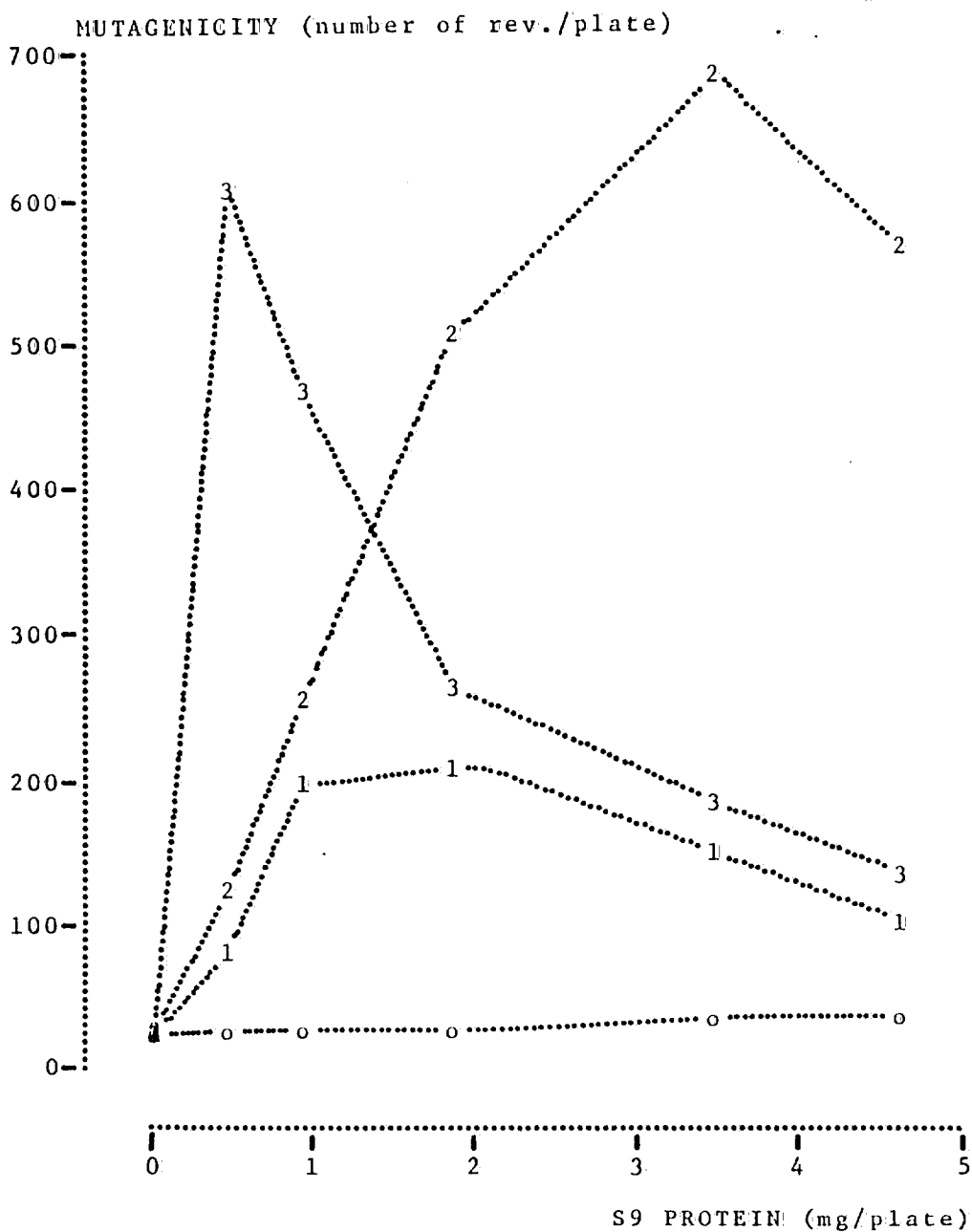


FIGURE 5

PROMUTAGEN ACTIVATION BY VARIOUS DOSES OF S9 PROTEIN,
BATCH NO. 81.A WITH STRAIN TA 98
(see TABLE 14)

- o: DMSO, 4o ul/plate (spontaneous reversion)
- 1: WSC-I of 2R1, o.1o mg dry condensate/plate
- 2: B(a)P, 5 ug/plate
- 3: 2-AF, 2 ug/plate

date of determination: 2.Sep.81

Source: <https://www.industrydocuments.ucsf.edu/docs/gsmm0000>

2026043010

A-/2059.00

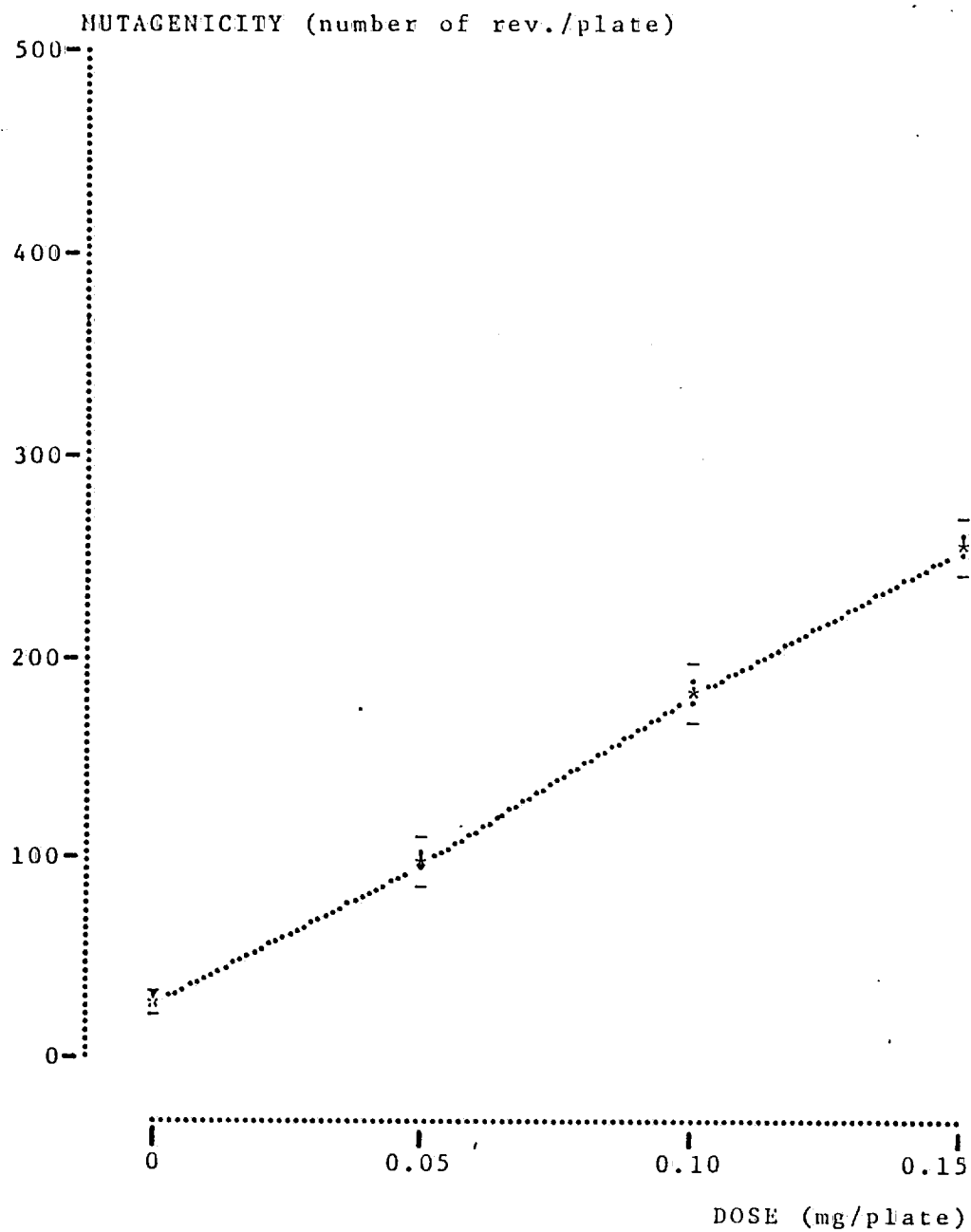


FIGURE 6

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 16)

2026049011

A-/2059.00

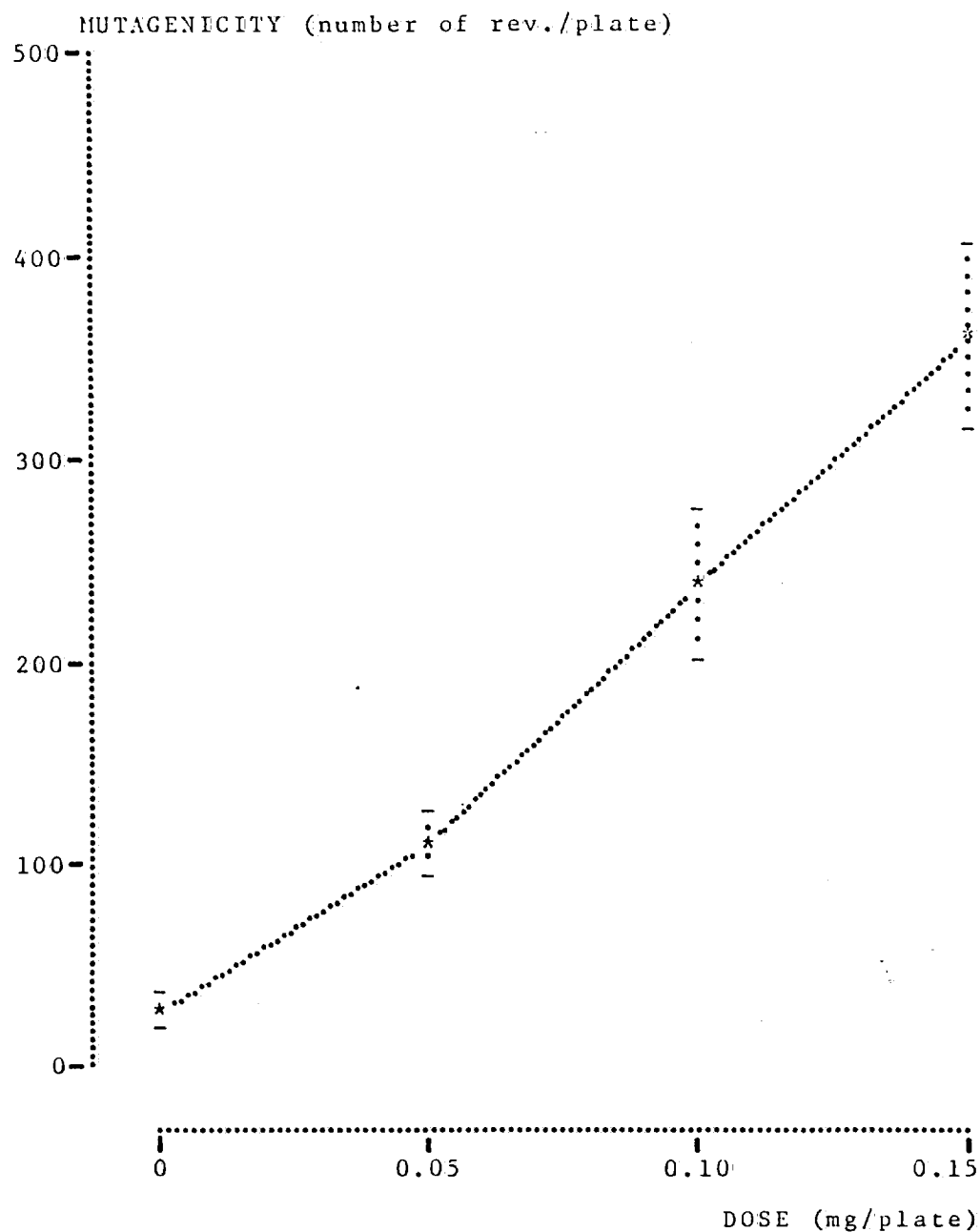


FIGURE 7

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 17)

2026043012

A-/2059.00

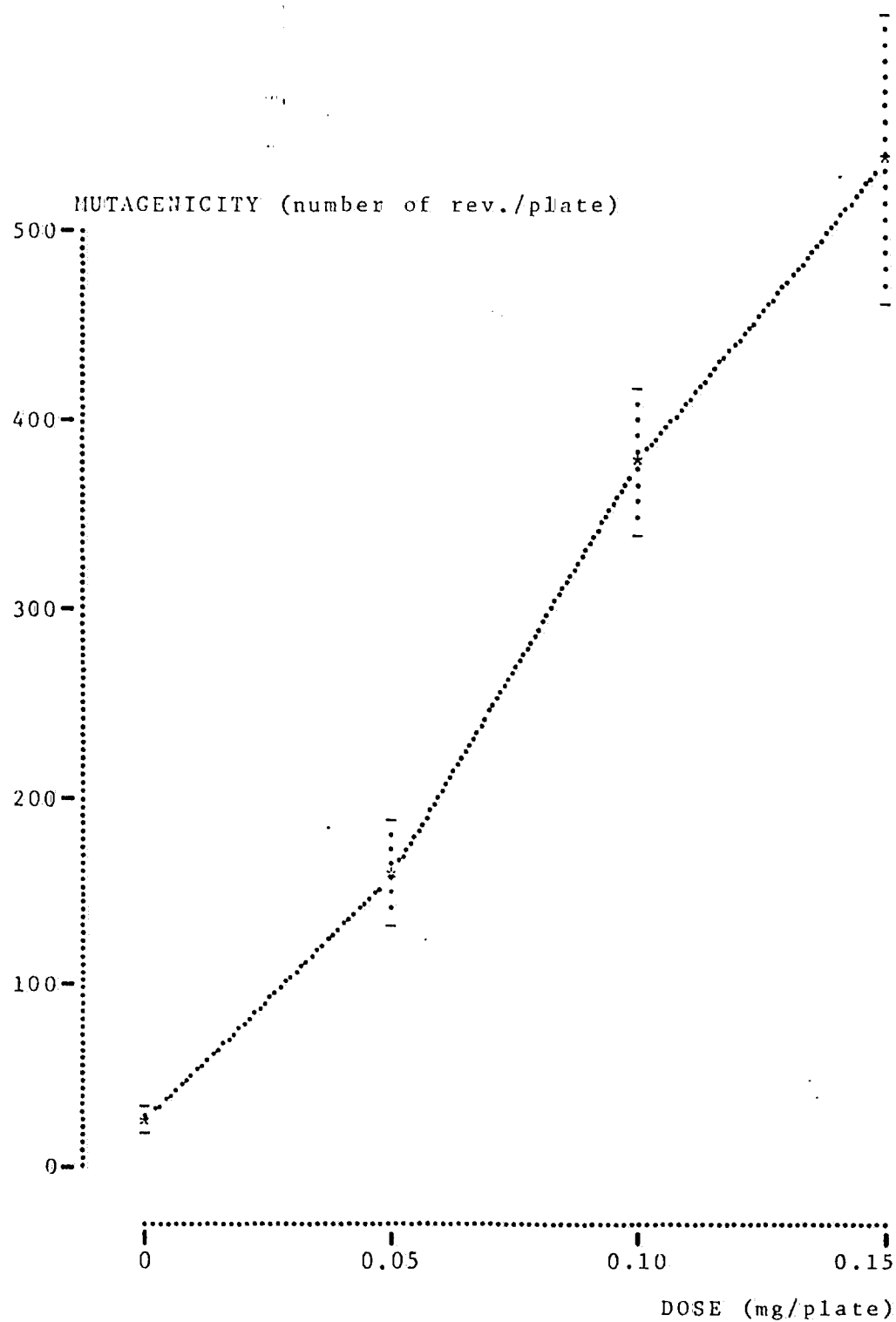


FIGURE 8

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 18)

2026043013

A-/2059.00

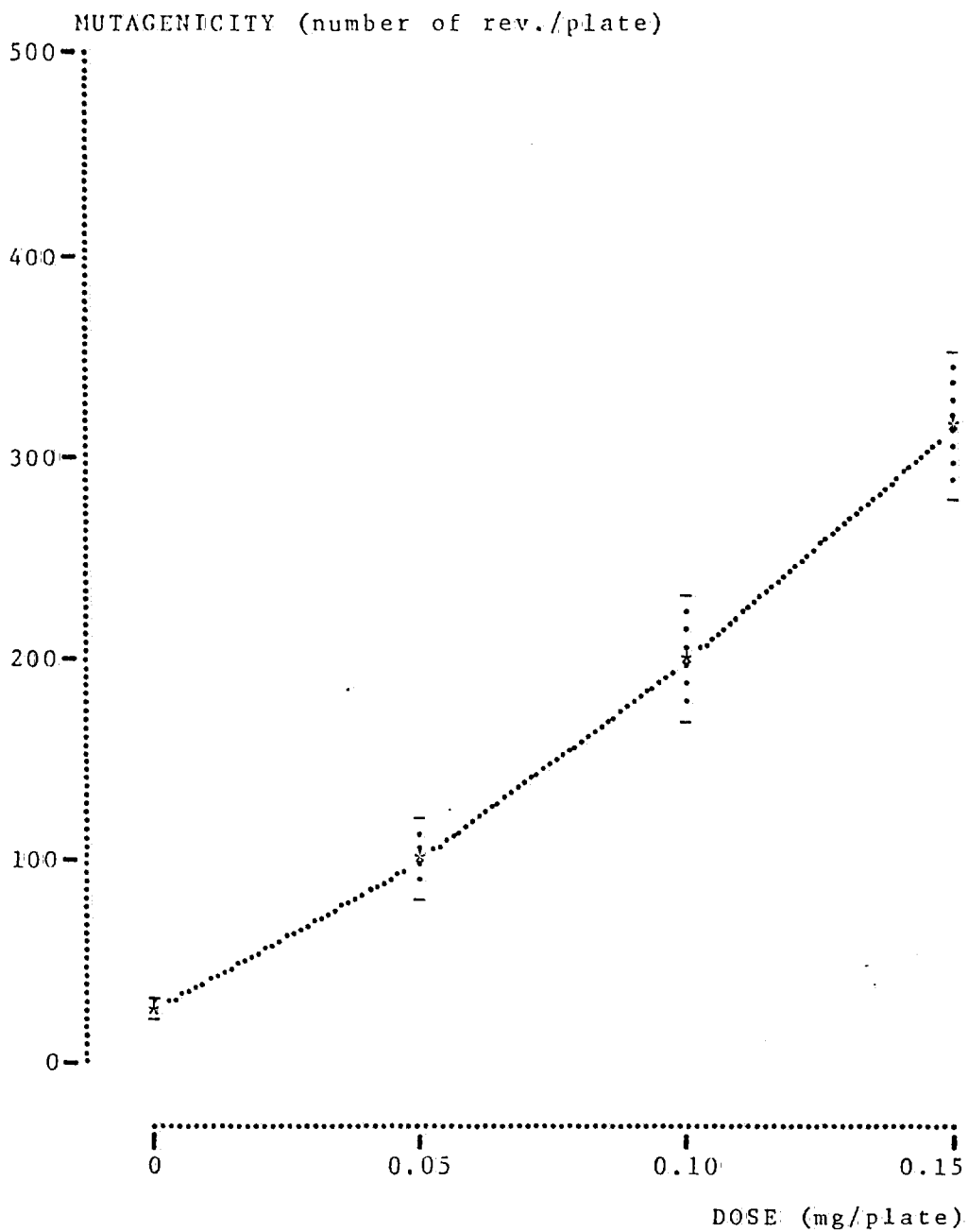


FIGURE 9

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 19)

2026043014

A-/2059.00

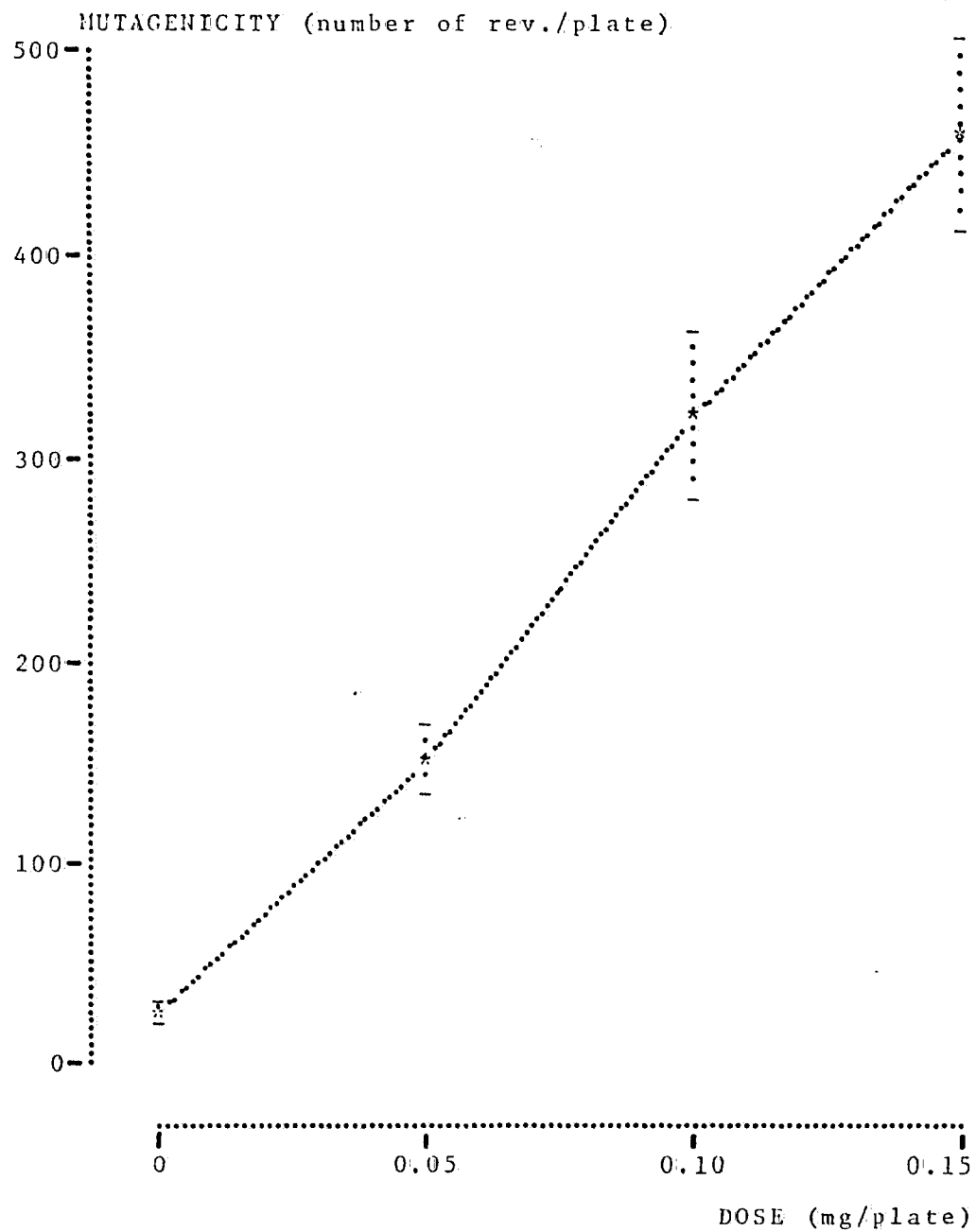


FIGURE 10

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 20)

2026043015

A-/2059,00

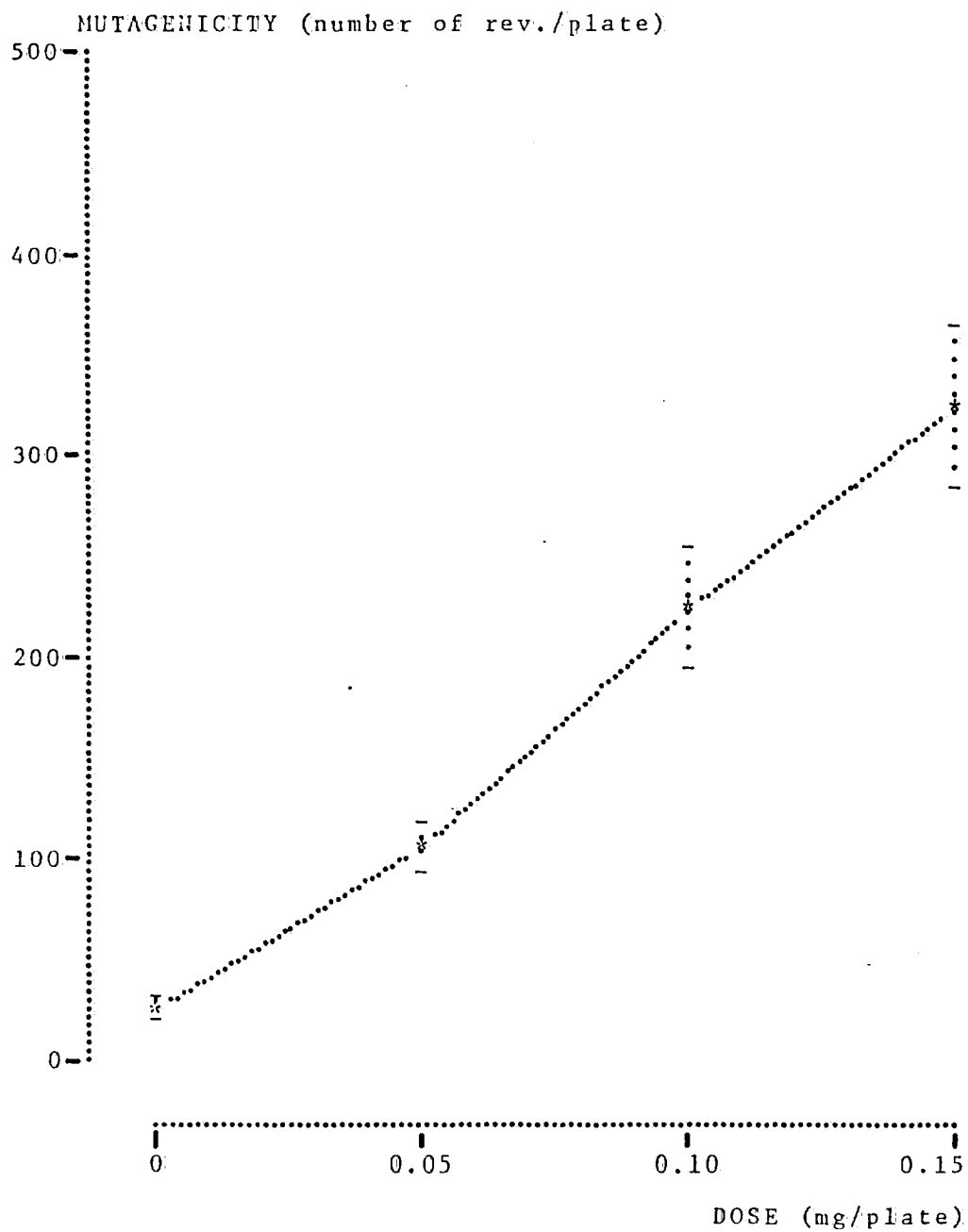


FIGURE 11

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 21)

2026043016

A-/2059.00

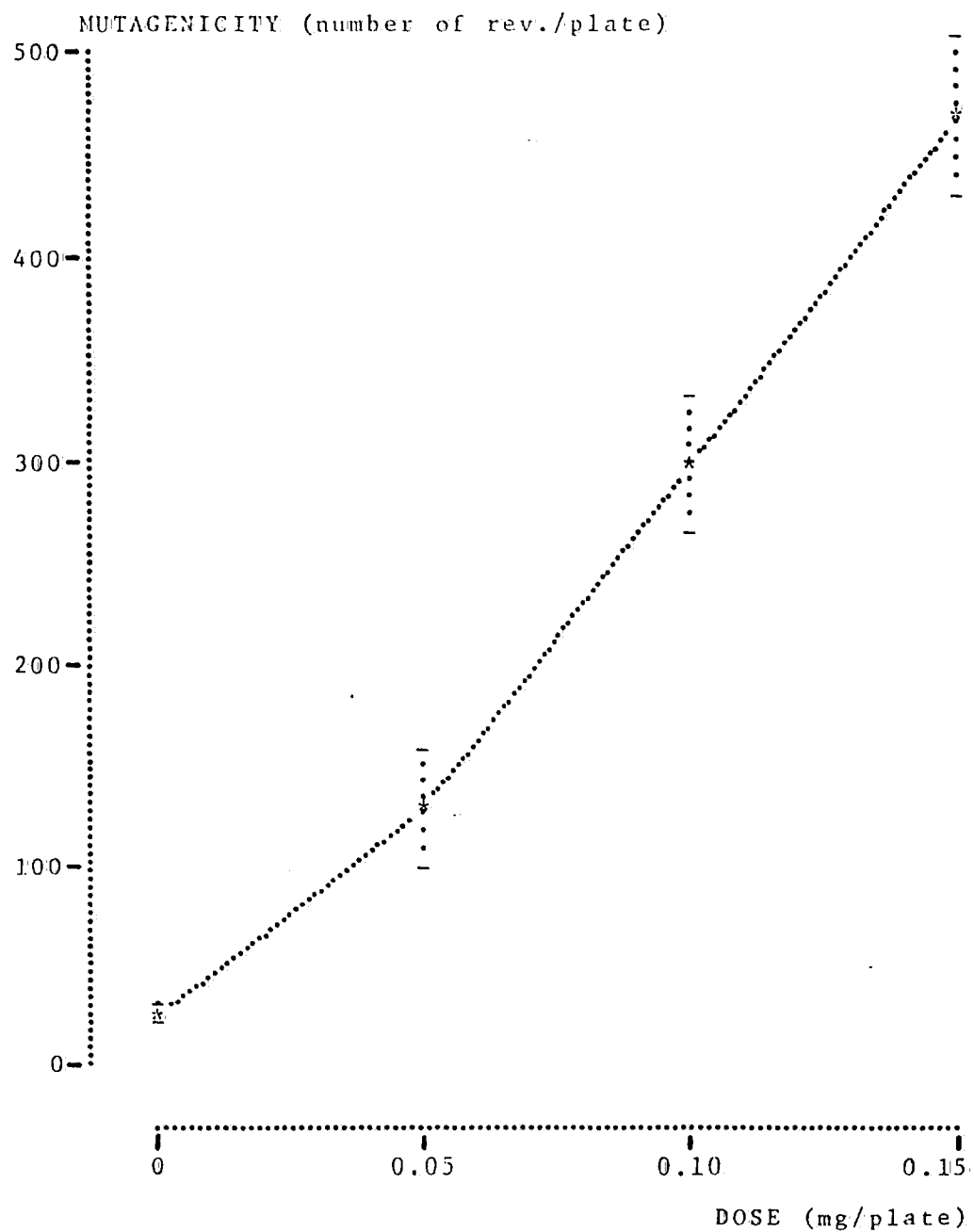


FIGURE 12

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 22)

2026049017

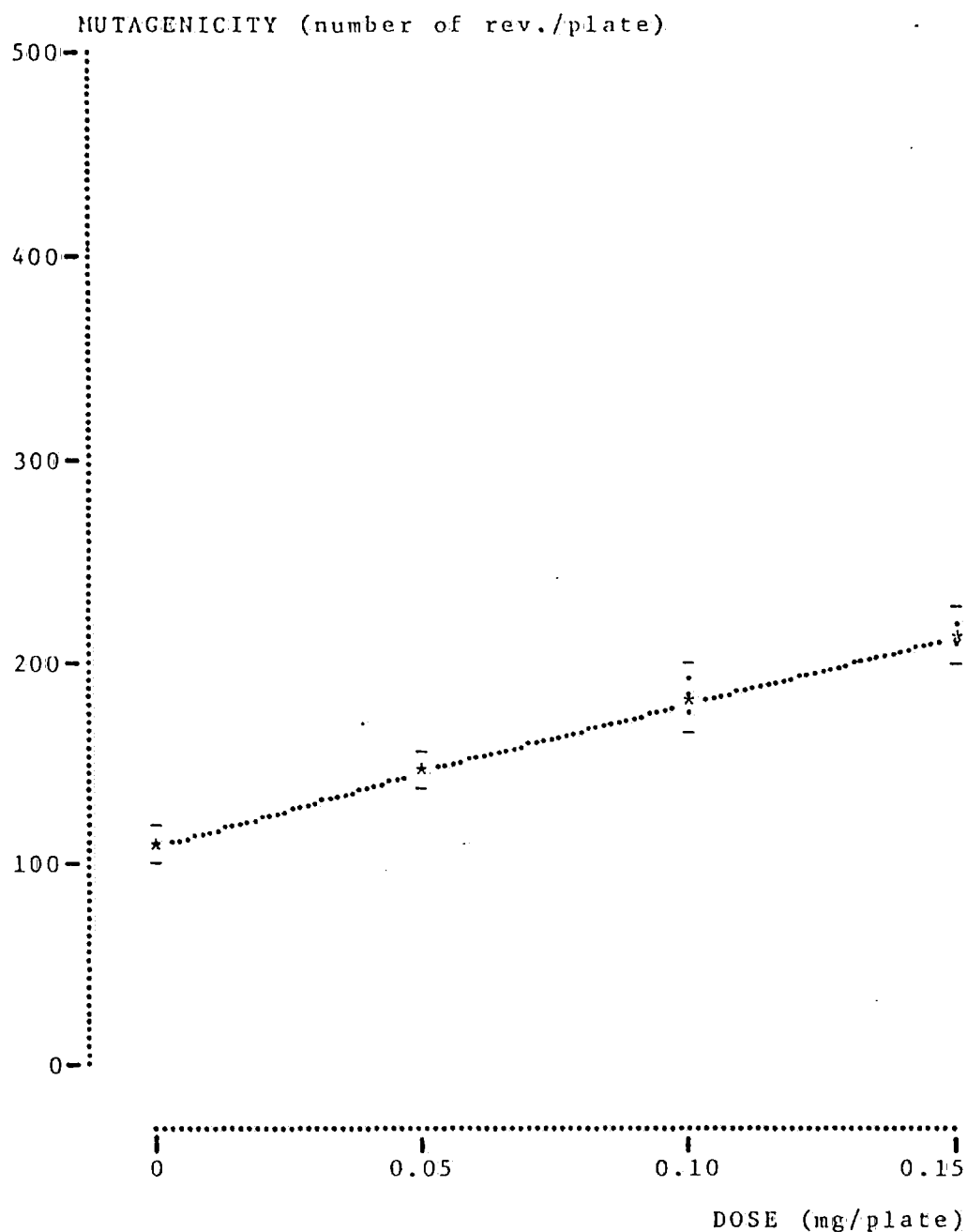


FIGURE 13

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 23)

A-/2059.00

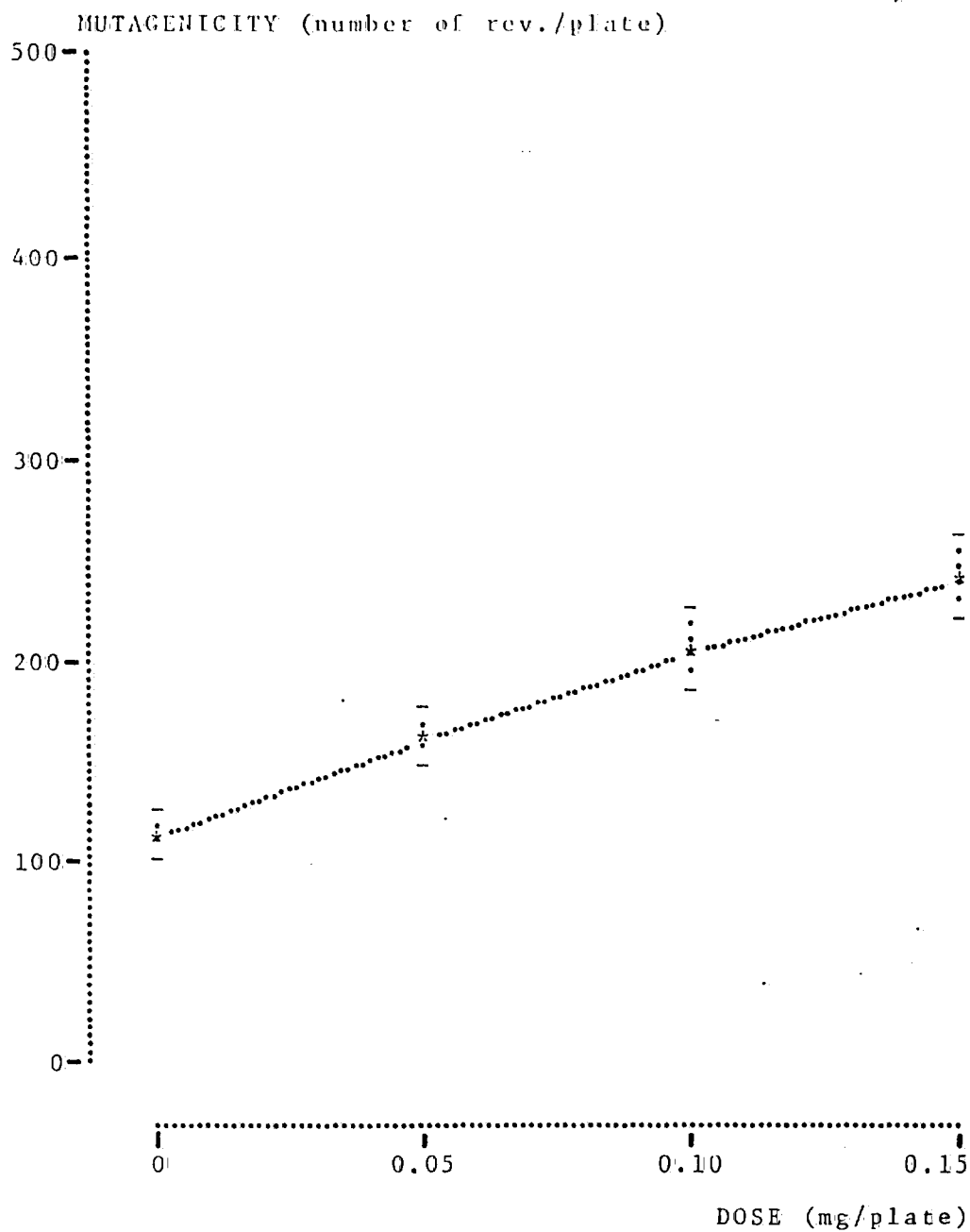


FIGURE 14

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 24)

2026049019

A-/2059.00

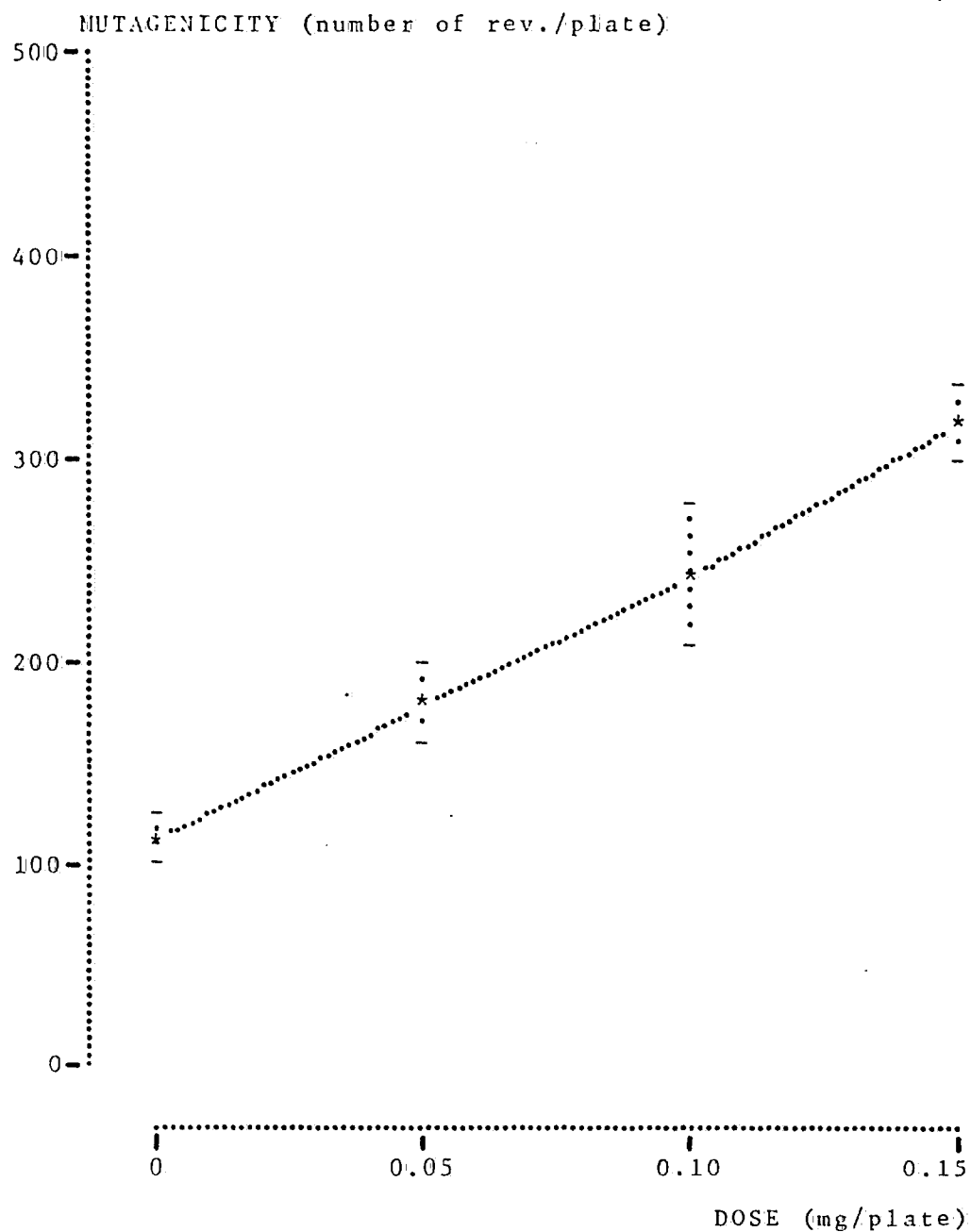


FIGURE 15

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 25)

2026049020

A-/2059.00

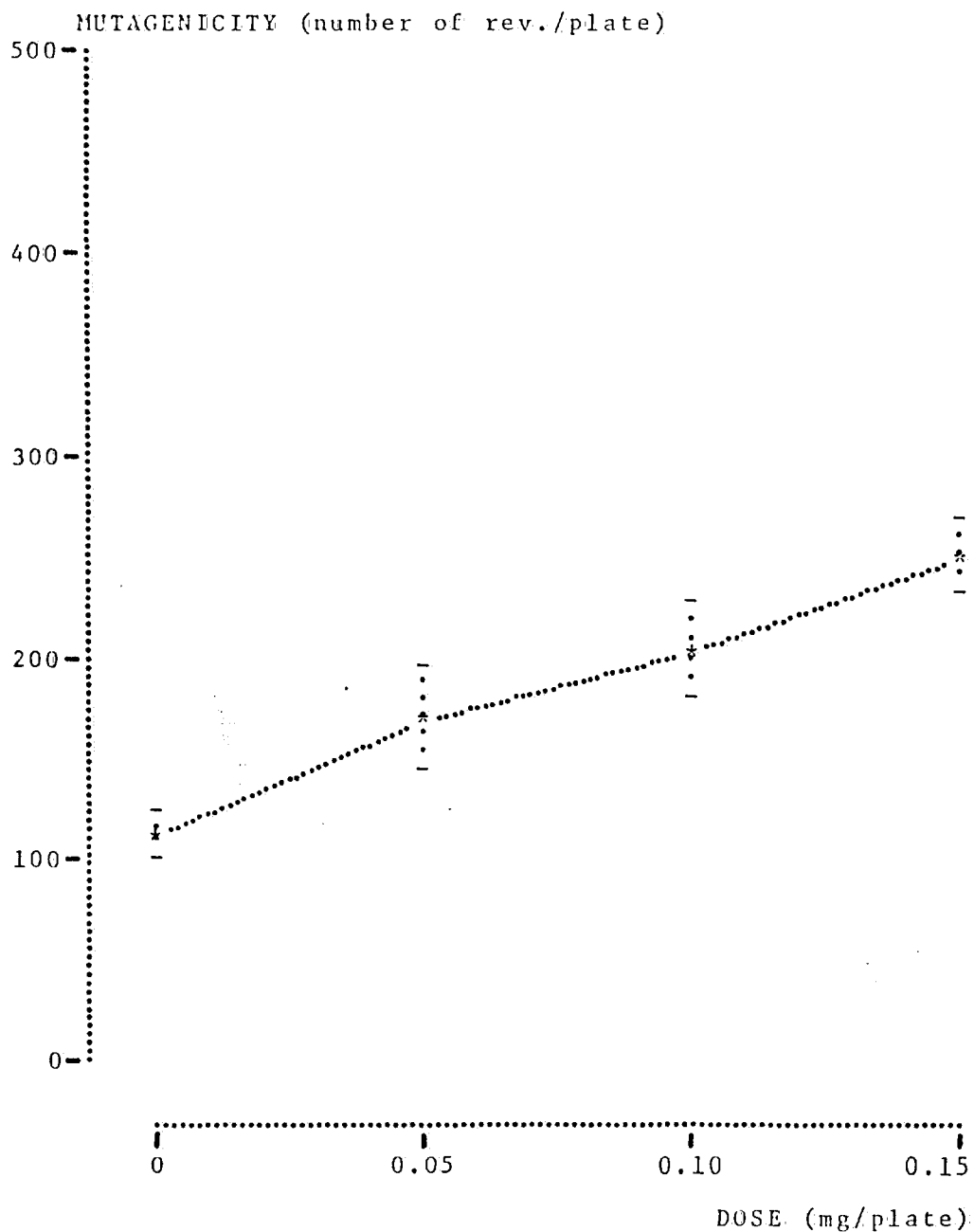


FIGURE 16

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 26)

2026043021

A-/2059.00

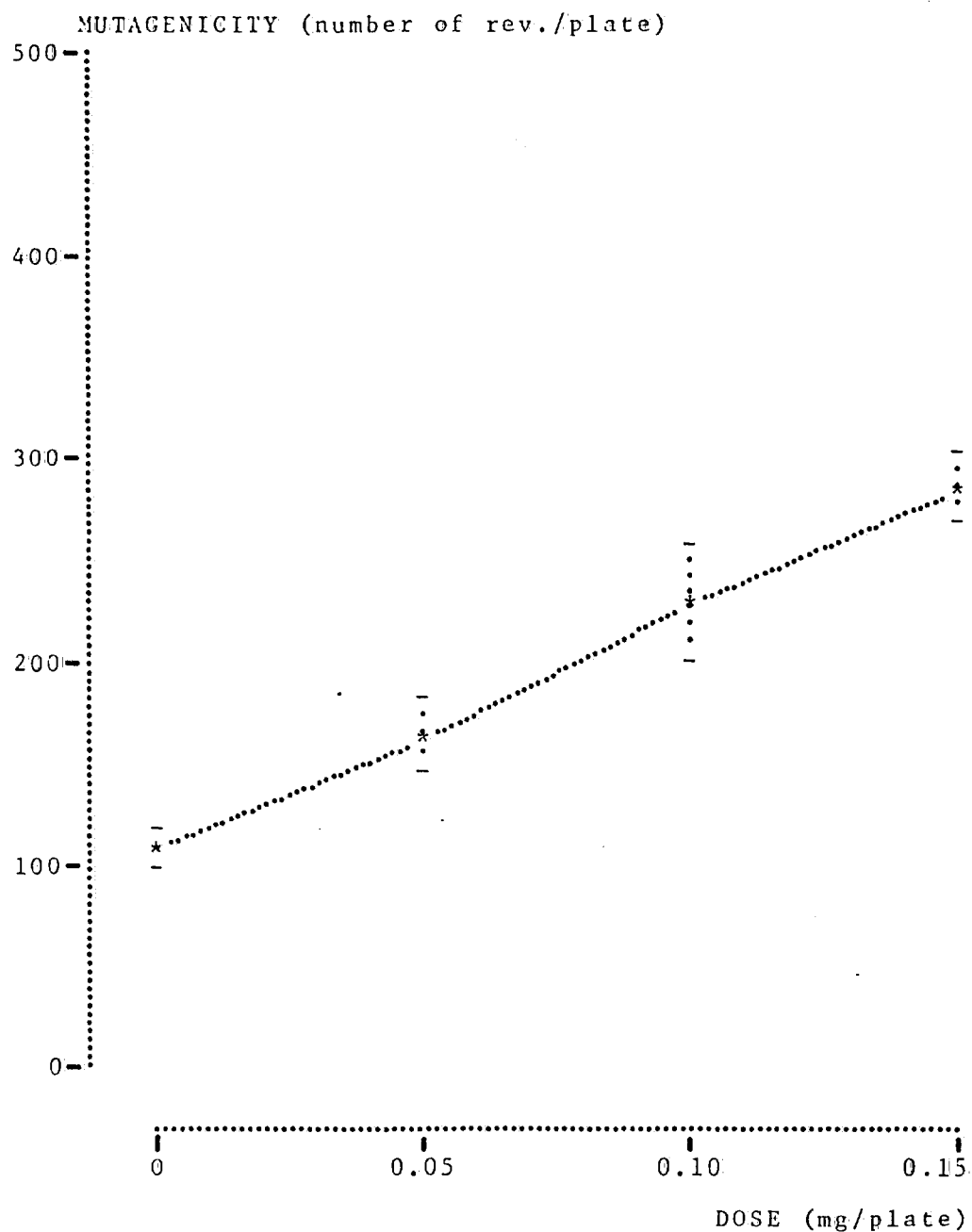


FIGURE 17

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 27)

2026049022

A-/2059.00

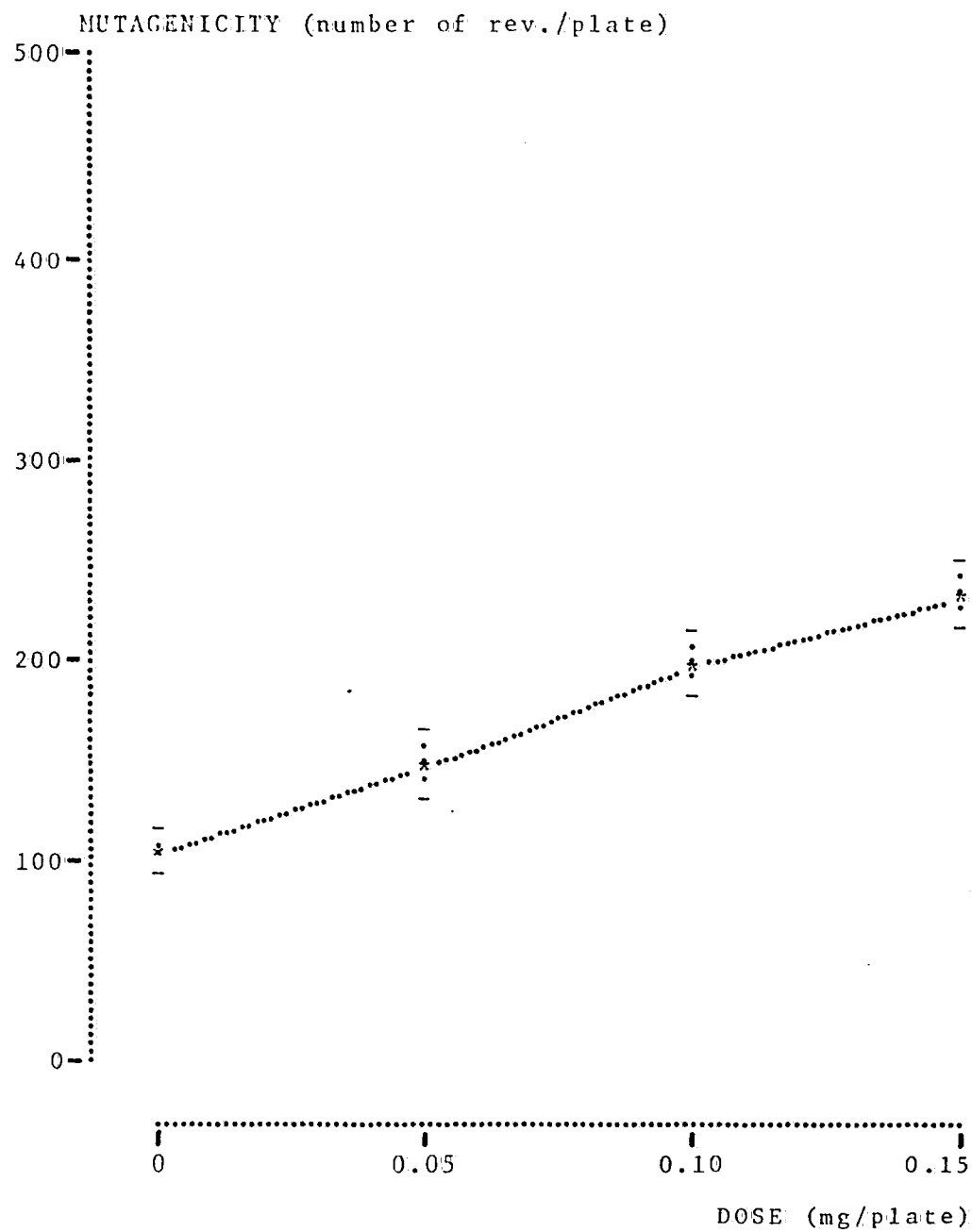


FIGURE 18

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 28)

2026049023

A-/2059.00

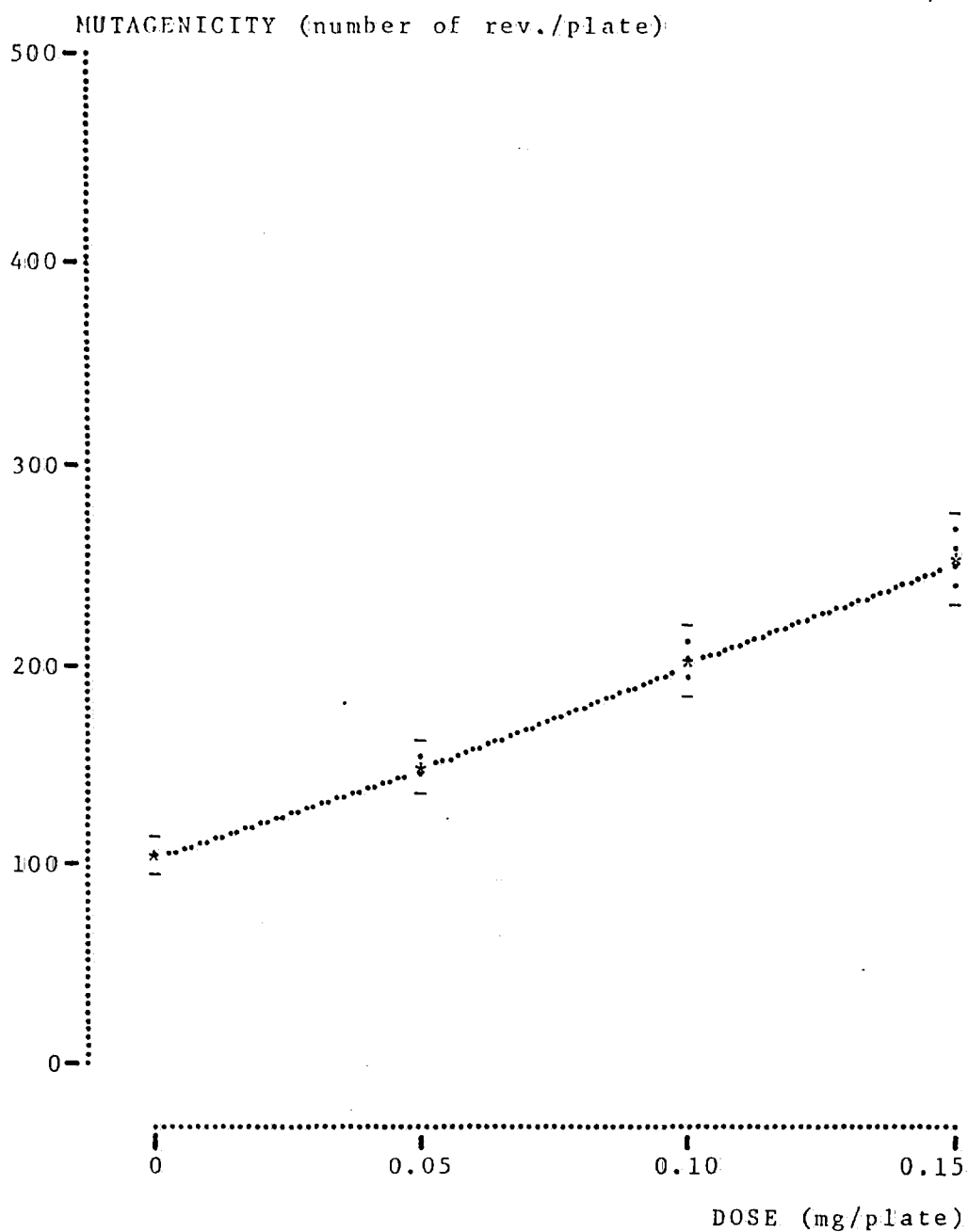


FIGURE 19

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 29)

2026049024

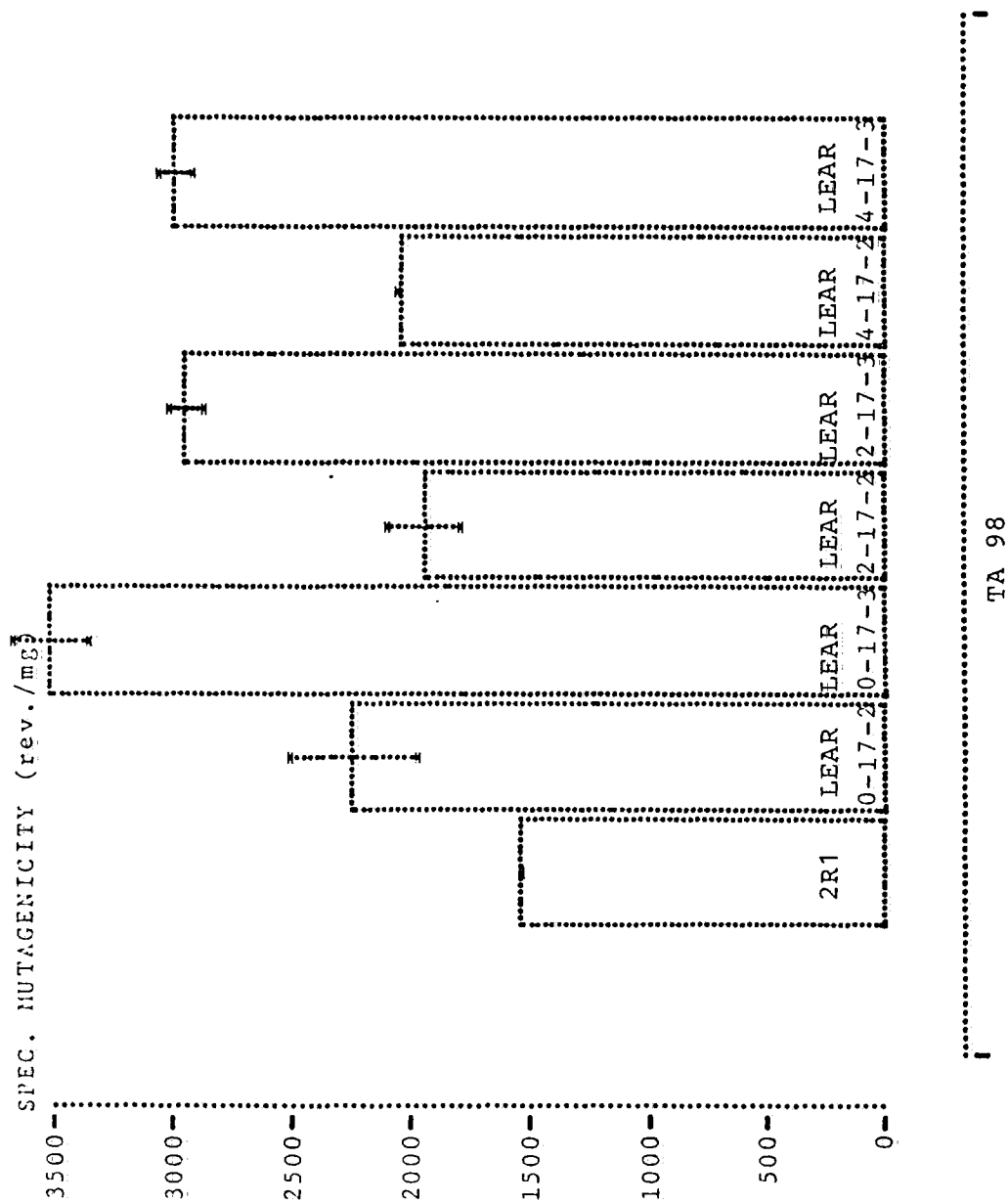


FIGURE 20
 SPECIFIC MUTAGENICITY OF CIGARETTES, STRAIN TA 98
 means of assay 1 and 2 with 64 plates
 (see TABLES 32 to 38)

2026049025

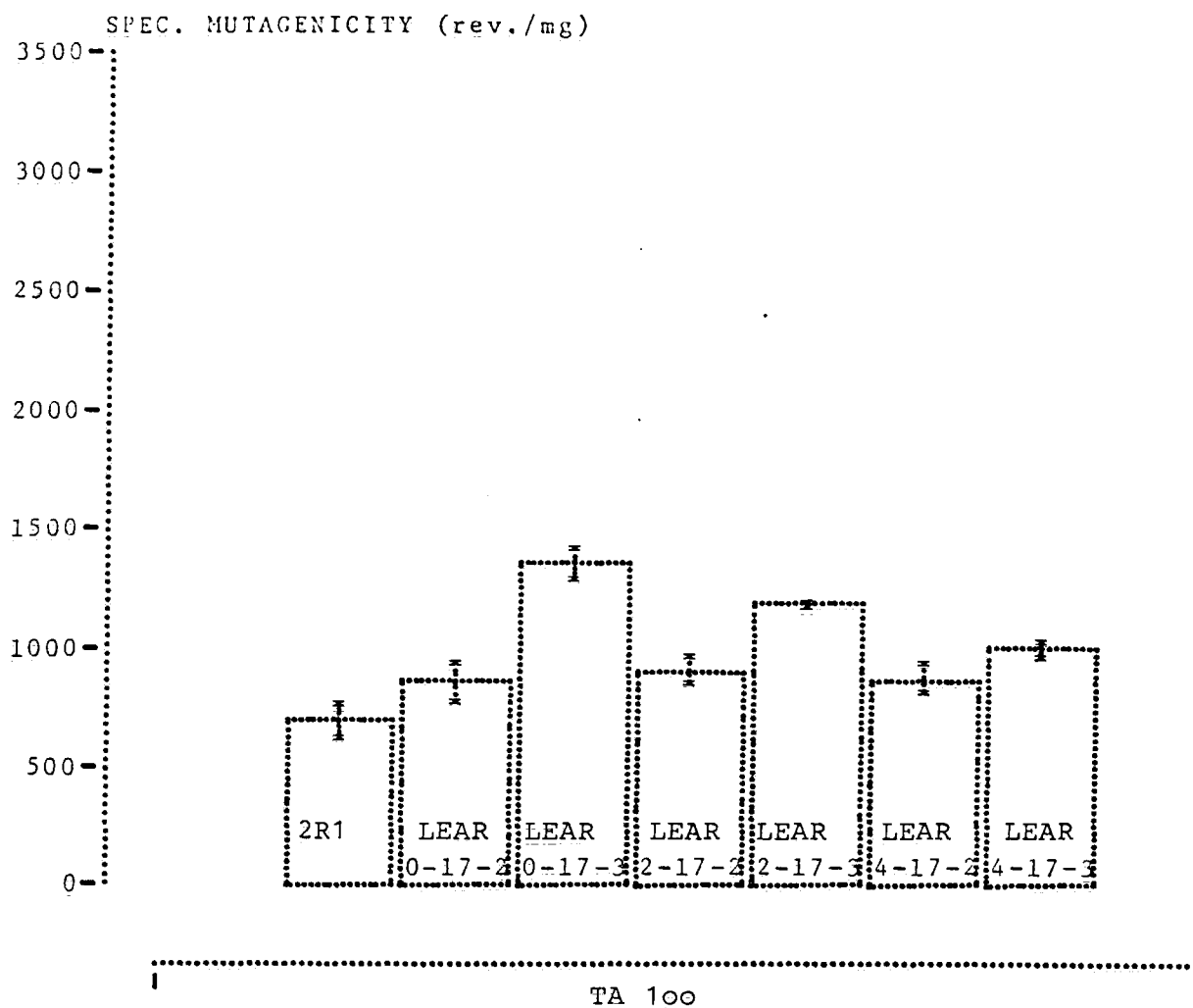


FIGURE 21

SPECIFIC MUTAGENICITY OF CIGARETTES, STRAIN TA 100

means of assay 1 and 2 with 64 plates
 (see TABLES 39 to 45)

2026049026

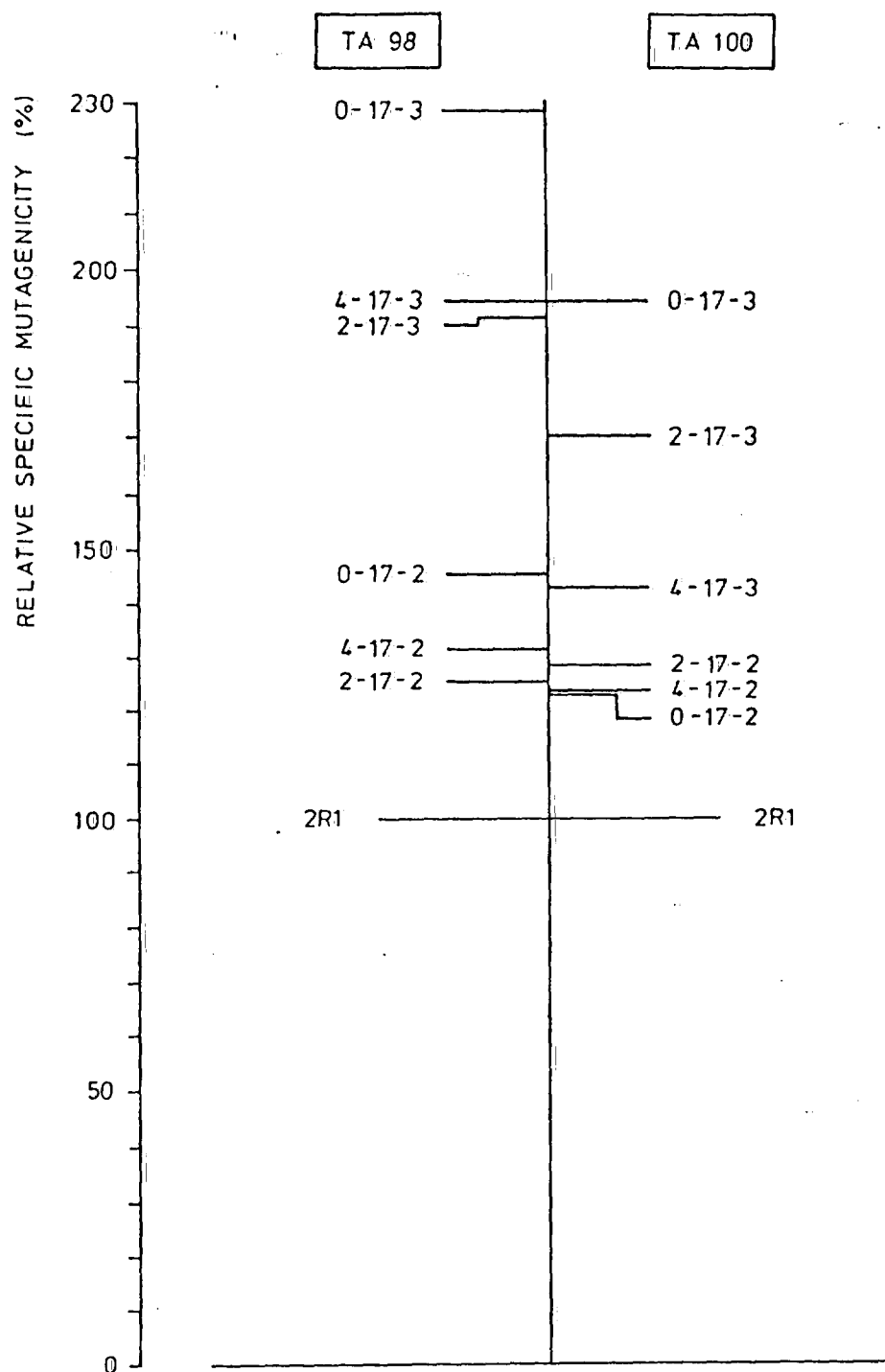


FIGURE 22

SPECIFIC MUTAGENICITY OF WSC-I OF "LEAR" CIGARETTES
RELATIVE TO WSC-I OF STANDARD REFERENCE CIGARETTES 2R1,
STRAINS TA 98 AND TA 100
(see TABLES 47 and 48)

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=====

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END OF REPORT

2026043028